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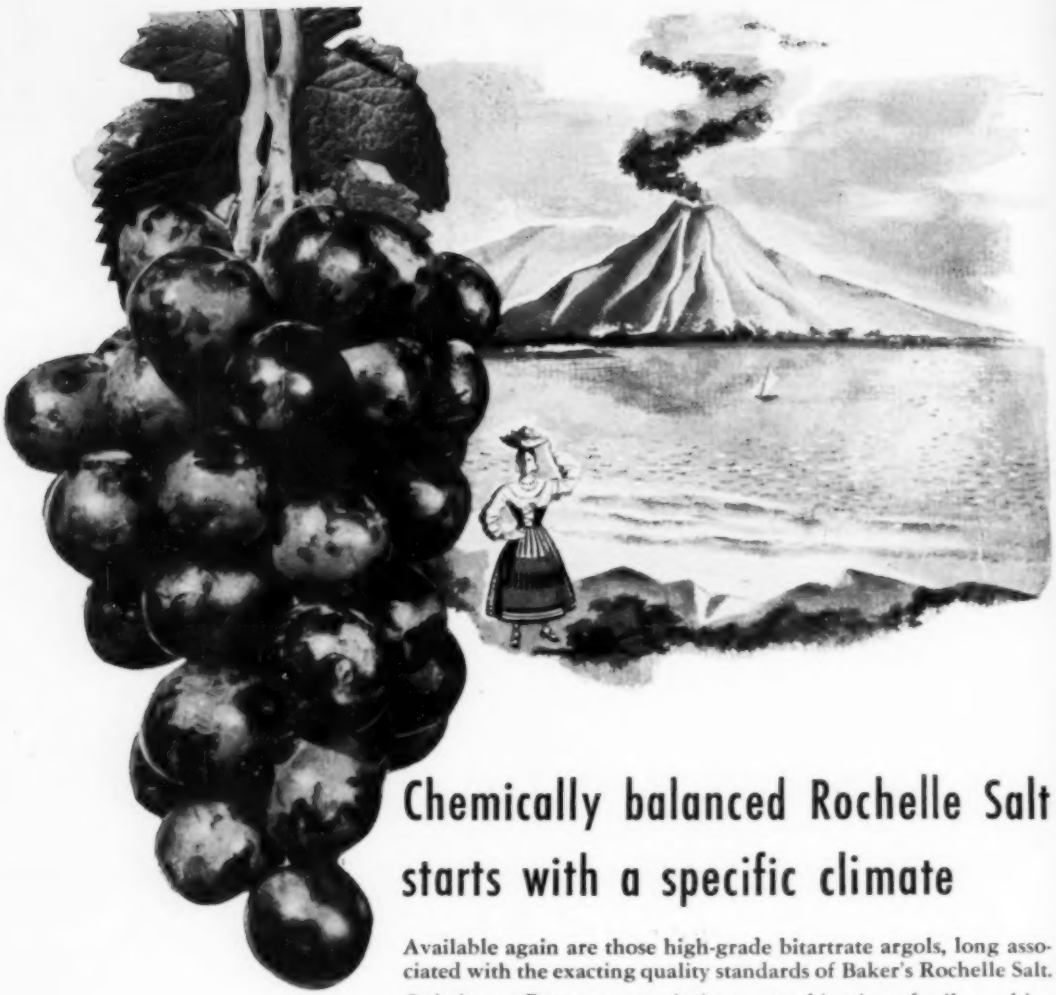

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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A New Concept of the Role of Hydrogen Ion Concentration and Buffer Systems in the Preparation of Ophthalmic Solutions*

By HARRY W. HIND† and FRANK M. GOYAN†

In this paper various buffer systems are listed and an analysis is made of their usefulness as pharmaceutical buffers. A report is also made of the results of a thorough study to determine the optimum *p*H level at which solutions of the more common ophthalmic drugs should be buffered in order to assume maximum stability and therapeutic efficiency. These ophthalmic drugs are then classified into five groups and five buffer systems are recommended to the pharmacist for compounding prescriptions calling for these drugs.

FOR SOME TIME there has been confusion concerning the selection of proper buffer systems and *p*H levels for the preparation of solutions of ophthalmic drugs (1–3). Gifford and Smith (1) stressed the physiological activity of ophthalmic drugs and indicated that many of the alkaloidal salts such as eserine, pilocarpine, and atropine were more effective in the alkaline region. Their solutions of these drugs, however, were prepared fresh at the time of instillation into the eye and little consideration was given to their chemical stability.

From the standpoint of the pharmacist, who is dispensing a prescription for a patient who will normally be using the medication for a period of from thirty to sixty days after it is filled, the problem of chemical stability as well as other factors becomes a matter of great importance. The need for more successful ophthalmic buffer solutions was pointed out by Hosford and Hicks (4) who realized the limitations of the systems offered by Gifford and Smith (1) as well as that offered by Feldman (5) and suggested the development of more suitable pharmaceutical buffers. In accordance with these suggestions of Hosford and Hicks (4), and to remove some of the existing confusion, we have undertaken a systematic study in an effort to develop more satisfactory solutions

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and to gain insight into the problem. In this work the following factors have been taken into consideration: buffer systems; buffer capacity; hydrogen ion concentration; osmotic effect; equilibrium concentration of the free base, and chemical stability. All of these factors were found to be interrelated when dealing with the administration of ophthalmic solutions containing physiologically active drugs. This is a new approach to the study of ophthalmic buffers because it integrates physical chemical principles with the fact that it is the drug action itself that is responsible for the stinging and burning sensation associated with many ophthalmic solutions. The effect of the *p*H on the equilibrium concentration of the free base of many different alkaloids is shown to be a factor of practical importance in the adjustment of ophthalmic solutions.

THEORETICAL DISCUSSION

It is generally thought that solutions to be instilled into the eye should have a *p*H equal to the normal *p*H of the tear (*p*H 7.4). This concept, however, requires qualification and should be slightly modified, especially when one considers these two points: (*a*) equilibrium concentration of the free base, and (*b*) the stability of the solution. In many cases these are more important considerations than the adjustment of *p*H isohydric with the tears.

Goyan and Daniels (6) suggested an approach to this problem by pointing out the type of dissociation and equilibria involved in solutions of salts of active bases. As a typical example the following equations show the equilibria encountered in a solution of pilocarpine hydrochloride.

HYDROLYSIS EQUILIBRIUM FOR PILOCARPINE HYDROCHLORIDE

$$\underset{\text{Pilocarpine Hydrochloride}}{C_{11}H_{16}N_2O_2HCl} \rightarrow \underset{\text{Pilocarpine Ion}}{C_{11}H_{16}N_2O_2H^+} + \underset{\text{Chloride Ion}}{Cl^-} \qquad \text{(I)}$$

$$\underset{\text{Pilocarpine Ion}}{C_{11}H_{16}N_2O_2H^+} + H_2O \rightleftarrows \underset{\text{Pilocarpine Base (Hydrated)}}{C_{11}H_{16}N_2O_2HOH} + \underset{\text{Hydrogen Ion}}{H^+} \qquad \text{(II)}$$

The equilibria described below may be applied generally to most physiologically active bases used in ophthalmic practice. It follows from equation II that the equilibrium concentration of the free base may be controlled by adjusting the hydrogen ion concentration. In general, neutral or slightly alkaline solutions favor high equilibrium concentration of the free base, while slightly acid solutions tend to reduce the amount of the free base. The concentration of the free base at various *p*H levels may be calculated from the constant of the base. Table I and Fig. 1 show the calculated mole per cent of free base in equilibrium with dilute water solutions of salts of various drugs used in ophthalmic work.

A high concentration of the free base of certain ophthalmic drugs has been found to be intensely irritating. We have found this irritation can be reduced by controlling the concentration of the free base by lowering the *p*H of the solution. On the other hand, there are many ophthalmic drugs that may be used advantageously at higher *p*H levels, because their active bases are not irritating to the eye tissue.

EFFECT OF *p*H

Aqueous solutions of acid salts of physiologically active bases are most stable at a *p*H from 2 to 3, because the ester linkages present in most of these bases can be expected to undergo hydrolysis and decomposition in neutral or alkaline solutions. As indicated in Equation II and Table I low *p*H values reduce the equilibrium concentration of the free bases and thereby retard physiological activity since it is usually the relative concentration of the free base and not that of the ionized salt that is correlated with the physiological effect (7a). The *p*H required for optimum activity may or may not correspond to the *p*H for optimum stability. If the two do not coincide it may be necessary to sacrifice optimum activity in order to obtain a stable ophthal-

TABLE I.—CALCULATED PERCENTAGE OF FREE BASE IN EQUILIBRIUM WITH THE SALTS OF VARIOUS ALKALOIDS

		Calculated Mole Per Cent of Free Base[a]				
Drug	*p*Kb[b]	*p*H 4	*p*H 6.8	*p*H 7.4 (Tears)	*p*H 8.0	*p*H 9.0
Atropine	4.35	0.0002	0.1	0.6	2.2	18.3
Cocaine	5.59	0.004	2.4	8.9	28.0	79.6
Dionin	6.12	0.013	7.7	24.9	56.9	93.0
Ephedrine	4.64	0.0004	0.3	1.1	4.2	30.4
Eserine	6.12	0.013	7 7	24.9	56.9	93.0
Pilocarpine	7.15	0.141	47.1	78.1	93.4	99.3
Procaine	5.15	0.001	0.9	3.4	12.4	58.5

[a] The value of 14.0 was taken for *p*Kw.
[b] The tabulated *p*Kb values given by Kolthoff (7) and others (6) were assumed to be correct at "room temperature."

mic solution. We have found that a *p*H level from 6.5 to 6.8 is satisfactory for buffering some ophthalmic drugs and that for others a *p*H value of 5 was necessary. In all cases we were able to obtain a high order of activity without materially sacrificing the chemical stability of the solution.

Not only has it been found that the eye can tolerate a wide change in osmotic pressure (8) when the *p*H is controlled, but can also tolerate without apparent irritation a wide change in *p*H value (8, 9) when the osmotic pressure is controlled. These observations are in accord with our findings that it is not necessary to maintain the *p*H of ophthalmic solutions at a value isohydric with tears. In fact, it is far more important to adjust the *p*H of these solutions so as to control within limits the initial equilibrium concentration of the irritating free bases associated with drugs listed below in Group 1, while at the same time making allowances for chemical stability and optimum physiological activity. If stability is of primary concern even the drugs listed below in Group 2 should be dispensed at a lower *p*H level (boric acid *p*H 5). In general it has been observed that nothing is gained by forming high concentrations of free base associated with solutions dispensed in the alkaline region as recommended by Gifford and Smith (1).

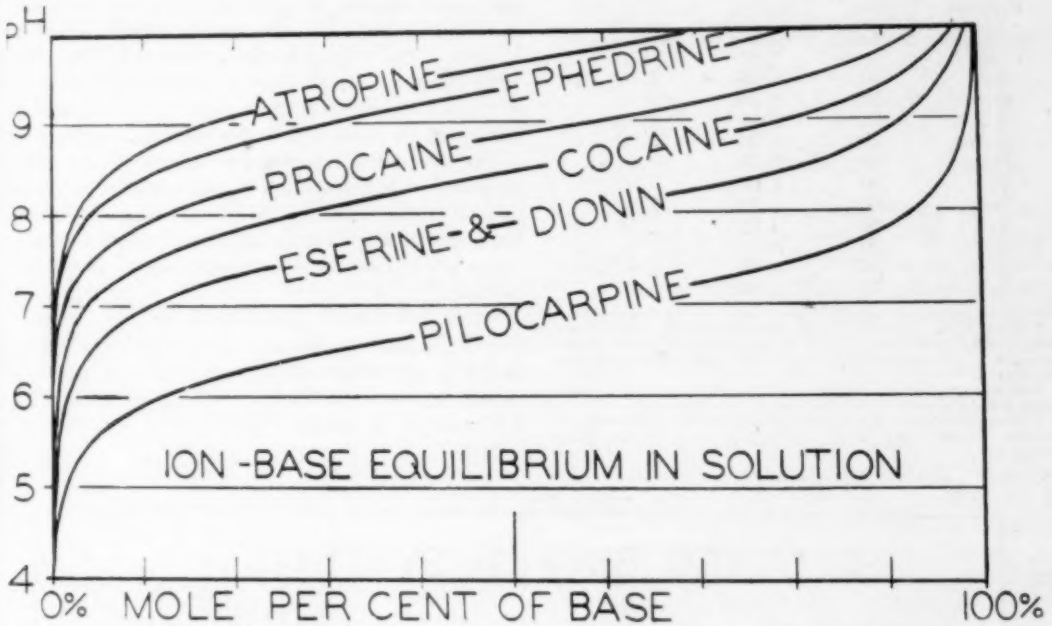

Fig. 1.—Ion Base Equilibrium in Solutions of Salts of Certain Ophthalmic Drugs.

OSMOTIC EFFECT

In the development of efficient ophthalmic buffers, osmotic pressure is recognized as one of the important factors to be considered. Freezing points of a series of contact lens solutions, judged to have optimum wearing qualities by the majority of a large number of patients, were determined according to a modified Hitchcock procedure (10). It was found that the tonicity of these solutions consisting of saline solutions, borate buffers and phosphate buffers, corresponded to a freezing point of −0.54°, which is the approximate freezing point of a physiological saline solution (U. S. P. XII). This is contrary to the generally accepted tonicity, corresponding to a 1.4% sodium chloride solution advocated for isotonic ophthalmic solutions. There is little valid evidence to support the assumption that a 1 4% solution of sodium chloride is isotonic with lacrimal fluid. Krogh, *et al.* (10*a*) recently published the value of 0.90% sodium chloride as the osmotic concentration of lacrimal fluid. According to the work on contact lens solutions by Hind (8) the eye has been shown to have a wide tolerance to solutions of varying osmotic pressure. Freezing point values of −0.331° to −1.244° or, in terms of sodium chloride equivalent of a 0.5% to 2% concentration, may be well tolerated. The instillation of one or two drops of a medicating eye drop has such a transitory osmotic effect on the eye tissue that for practical purposes the osmotic factor need not be accurately adjusted as long as it is maintained within reasonable physiological limits.

BUFFER SYSTEMS

Because hydrogen ion concentration is one of the most important factors influencing the effects of ophthalmic solutions, a consideration of proper buffer systems is, therefore, important. Buffer solutions differ considerably with respect to buffer capacity which is a function of the concentration

and *p*H range of the buffer system used. The importance of buffer capacity in studying such solutions is frequently overlooked. In some cases it may be desirable to have a low buffer capacity while in others, where certain pathological conditions exist, current opinion suggests the use of solutions having maximum buffer capacity. Figure 2 shows the relationship between *p*H and buffer capacity for several buffer systems. The regions of high buffer capacity correspond to the plateaus of the curves. The following is a list of common buffer solutions that have been modified for use in ophthalmic practice. These are listed so that we may discuss their usefulness, as well as their limitation.

TABLE II.—A REVALUATION OF THE *p*H VALUES OF THE GIFFORD BUFFERS

Boric Acid Solution		Boric Acid[a]	12.4 Gm.
		Potassium Chloride	7.4 Gm.
		Distilled Water	1000 cc.
Sodium Carbonate Solution		Sodium Carbonate Anhydrous[b]	21.2 Gm.
		Distilled Water	1000 cc.
Reported Value by Gifford, *p*H	Boric Acid Solution, Cc.	Sodium Carbonate Solution, Cc.	Reported Value by Hind and Goyan, *p*H 25.5°[c]
5.0	30	0.00	4.66[a]
6.0	30	0.05	5.99
6.2	30	0.10	6.24
6.75	30	0.25	6.62
6.95	30	0.50	6.91
7.2	30	1.00	7.23
7.6	30	1.50	7.42
7.8	30	2.00	7.58
8.2	30	3.00	7.81
8.4	30	4.00	7.97
9.0	30	8.00	8.47

[a] Boric Acid, c. p., dried over calcium chloride to constant weight and dissolved in U. S. P. distilled water with potassium chloride. This solution has a very low buffer capacity in this *p*H region.
[b] Sodium carbonate anhydrous, c. p., dried at 120° to constant weight and dissolved in U. S. P. distilled water.
[c] Measurements made with the *p*H meter described by Goyan, Barnes, and Hind (11) using the Coleman glass electrode.

Table II is an illustration of the buffer system used by Gifford (2) which is a slight modification of the alkaline buffer mixture developed by Atkins and Pantin (12) and has commonly been referred to as the "Gifford Buffer." This buffer mixture when used below a *p*H of 8 has a marked tendency to become more alkaline on standing due to the fact that the solution gives off more carbon dioxide than it absorbs. The original workers (Atkins and Pantin, 12) recommended this system to be used between *p*H 8 and 10, which is the correct range for such a buffer system when high buffer capacity is essential.

The *p*H values reported by Gifford (2) are only approximate in the alkaline region as shown by comparison of the values in Table II. Figure 2 shows the orignal Gifford values and the revaluated values which are in close agreement with Atkins and Pantin (12).

Table III lists a modification of the Atkins-Pantin Buffer mixture (12) which has been found useful in buffering contact lens solutions, *p*H 7.8 to *p*H 8.4, as a solvent for soluble fluorescein (*p*H 8.4) and as an alkaline buffer for a therapeutic collyria.

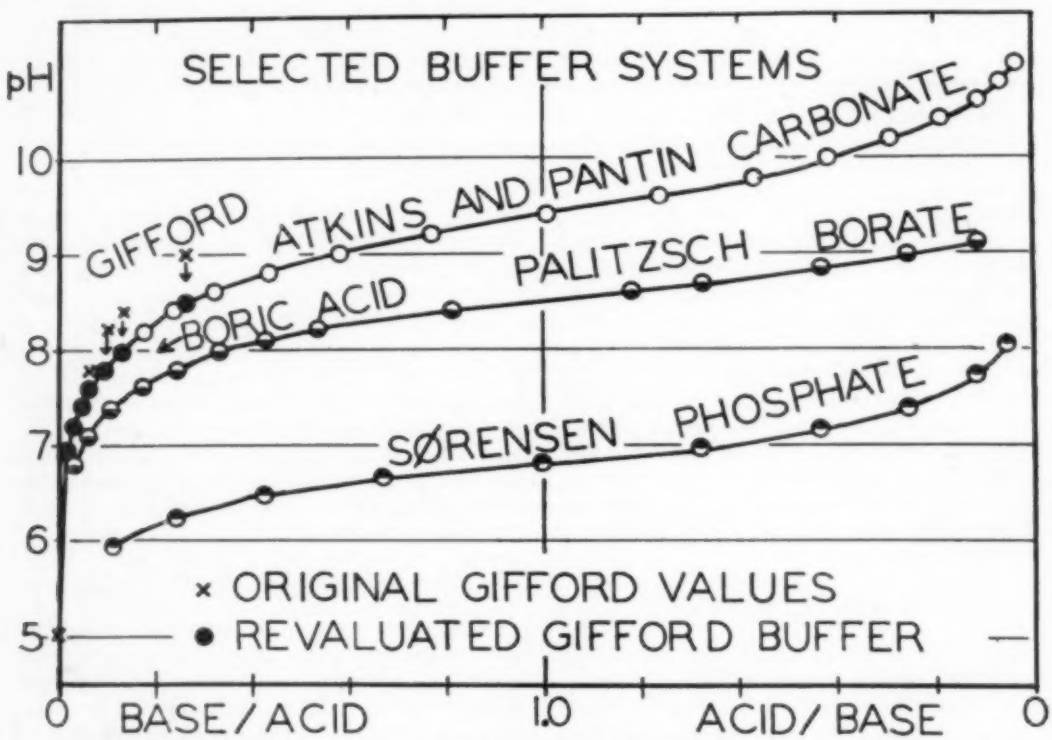

Fig. 2.—Buffer Systems Selected for Use in Connection with the Preparation of Ophthalmic Solutions.

TABLE III.—MODIFIED ALKALINE BUFFER MIXTURES (ATKINS AND PANTIN)

0.2 *M* Boric Acid Solution	Boric Acid, c. p.	12.405 Gm.
	Sodium Chloride	7.5 Gm.
	Distilled Water, q. s.	1000 cc.
0.2 *M* Sodium Carbonate Solution	Sodium Carbonate Anhydrous	21.2 Gm.
	Distilled Water, q. s.	1000 cc.

Boric Acid Solution, Cc.	Sodium Carbonate Solution, Cc.	*p*H (16°)
93.8	6.2	7.6
91.7	8.3	7.8
88.8	11.2	8.0
85.0	15.0	8.2
80.7	19.3	8.4
75.7	24.3	8.6
69.5	30.5	8.8
63.0	37.0	9.0
56.4	43.6	9.2
49.7	50.3	9.4
42.9	57.1	9.6
36.0	64.0	9.8
29.1	70.9	10.0
22.1	77.9	10.2
15.4	84.6	10.4
9.8	90.2	10.6
5.7	94.3	10.8
3.5	96.5	11.0

TABLE IV.—MODIFIED PALITZSCH BORATE BUFFER SYSTEM ADJUSTED TO BE ISOTONIC TO PHYSIOLOGICAL SALINE

0.05 *M* Sodium Borate Solution	Sodium Borate 10 H_2O	19.108 Gm.
	Distilled Water	1000 cc.
0.2 *M* Boric Acid Solution	Boric Acid	12.404 Gm.
	Distilled Water, q. s.	1000 cc.

*p*H	0.2 *M* Boric Acid, Cc.	0.05 *M* Borax, Cc.	Grams of Sodium Chloride per 100 Cc. to Render Isotonic
6.77	97.0	3.0	0.22
7.09	94.0	6.0	0.22
7.36	90.0	10.0	0.22
7.60	85.0	15.0	0.23
7.78	80.0	20.0	0.24
7.94	75.0	25.0	0.24
8.08	70.0	30.0	0.25
8.20	65.0	35.0	0.25
8.41	55.0	45.0	0.26
8.60	45.0	55.0	0.27
8.69	40.0	60.0	0.27
8.84	30.0	70.0	0.28
8.98	20.0	80.0	0.29
9.11	10.0	90.0	0.30

Table IV illustrates the Palitzsch Borate Buffer System (13) adjusted to be isotonic to physiological saline by the method of Goyan, Enright and Wells (14). This is an excellent borate buffer and is more dependable and stable than that advocated by Atkins and Pantin (12) because it does not contain carbonate. The Feldman (5) buffer is derived from the Palitzsch system and presents no outstanding advantages and several minor disadvantages because each solution of the Feldman series has a different volume. The modified Palitzsch buffer has a buffer range between *p*H 8 to 10 and is valuable pharmaceutically for buffering contact lens solutions *p*H 8 to 8.8 (8), as a vehicle for ophthalmic drugs, and as an alkaline collyrium. It is, of course, not suitable for solutions to be used parenterally.

TABLE V.—MODIFIED PHOSPHATE BUFFER SYSTEM (SØRENSEN) ADJUSTED TO BE ISOTONIC TO PHYSIOLOGICAL SALINE

M/15 Sodium Acid Phosphate	Sodium Acid Phosphate Anhydrous	8.006 Gm.
	Distilled Water, q. s.	1000 cc.
M/15 Disodium Phosphate	Disodium Phosphate Anhydrous	9.473 Gm.
	Distilled Water, q. s.	1000 cc.

*p*H	*M*/15 NaH_2PO_4, Cc.	*M*/15 Na_2HPO_4, Cc.	Grams of Sodium Chloride per 100 Cc. to Render Isotonic
5.91	90.0	10.0	0.38
6.24	80.0	20.0	0.39
6.47	70.0	30.0	0.40
6.64	60.0	40.0	0.41
6.81	50.0	50.0	0.42
6.98	40.0	60.0	0.44
7.17	30.0	70.0	0.45
7.38	20.0	80.0	0.46
7.73	10.0	90.0	0.47
8.04	5.0	95.0	0.48

Table V illustrates a modification of Sørensen's (15) phosphate buffer system. This system has a buffer range of *p*H 5.8 to 8, with maximum buffer capacity at *p*H 6.7. This is an efficient buffer solution for many opthalmic drugs, as it controls *p*H at the isohydric point of tears, and is a buffer system which contains no ions foreign to the eye. Some of the limitations of the phosphate buffer system are chemical incompatibility with zinc salts and a tendency to support mold growth.

Gershenfeld (16) and more recently Arrigoni (17) have recommended the use of esters of *para*-hydroxy benzoate for the prevention of mold growth in ophthalmic solutions. We have found that effective concentrations of these esters were found to produce a stinging sensation when applied to the eye. For several years we have successfully used alkyl dimethyl benzyl ammonium chloride[1] in a concentration of 1:50,000, and found it to be perfectly satisfactory in preventing mold growth in ophthalmic solutions.

DRUGS COMMONLY USED IN OPHTHALMIC PRACTICE

The drugs commonly used in opthalmic solutions have been assigned to groups according to the hydrogen ion level at which they are best buffered. The following solutions are recommended to be prepared as stock vehicles. The ophthalmic prescription is dispensed by weighing out the prescribed amount of active ingredient and dissolving in the suggested vehicle for the particular drug. Group 1 includes those drugs dispensed in a weak acid solution (*p*H 5). At this *p*H level they showed suitable stability, and satisfactory therapeutic properties such as prompt action with minimum irritation to the eye tissue.

[1] Zephiran Chloride manufactured by Winthrop Chemical Co. or Phemerol Aqueous, Parke Davis Co.

Group 1—Ophthalmic Drugs

Salts of:

Adrenalin	Nupercaine	Procaine
Cocaine	Optochin	Prostigmine
Dionin	Phenacaine	Syntropan
Metycaine	Pontocaine	Zinc

When drugs of this group are dispensed at the *p*H of the eye (7.4) precipitation of the free base has been observed in 1% solution of phenacaine hydrochloride, pontocaine hydrochloride, optochin hydrochloride, and nupercaine hydrochloride. In the case of epinephrine hydrochloride, dionin hydrochloride, syntropan phosphate, metycaine hydrochloride, prostigmine and cocaine where the base does not precipitate, the resulting solutions can be expected to be unstable and irritating due to the high concentration of free base in equilibrium with the salt. In all cases when the hydrogen ion concentration was lowered to *p*H 5 with a weak acid (boric) the solutions remained chemically stable and insured maximum therapeutic efficiency.

It was observed on groups of normal individuals that a lowering of *p*H to approximately 5 reduces the irritation associated with the initial instillation of solutions of drugs of Group 1 (except zinc salts) into the eye, and that the stinging and burning sensations become intense as the *p*H of the solutions is increased to neutrality (*p*H 7.0). This is in accordance with the fact that it is the free base that is primarily responsible for the burning. Equation II shows that the concentration of free base increases with the *p*H thus accounting for the correlation. It is obvious that many of these drugs are local anesthetics and the burning sensation is felt only until the onset of anesthesia. When dispensing drugs in this group a slightly acid solution should be used. It is important to note that simple water solutions rendered isotonic with sodium chloride when used as a vehicle for Group 1 did not prove to be very useful. This is probably due to the immediate neutralization of the solution when instilled into the eye; whereas, by maintaining a *p*H of approximately 5 with weakly dissociated acid this rapid neutralization is retarded sufficiently to allow a more prolonged and more gentle action. In this way the eye actually participates in the neutralization thereby slowly liberating the free base for absorption. Boric acid was found to be ideal for this purpose because it lowers the *p*H with very low buffer action. The following vehicle is therefore recommended for dispensing solutions of the drugs in Group 1.

SOLUTION FOR DISPENSING OPHTHALMIC DRUGS OF GROUP 1

Boric Acid, c. p.	0.356 *M*
Alkyl dimethyl benzyl ammonium chloride	1–50,000
Distilled Water	q. s.

Zinc Salts.—Zinc salts were included in Group 1 because solutions at *p*H values much above *p*H 6 have a tendency to form basic complexes of zinc, thereby reducing the zinc ion concentration. The work of Kolthoff and Kameda (18) showed that 0.01 *M* zinc sulfate solution will form basic hydroxides at a *p*H 6.49. The solution recommended for use with drugs in Group 1 proved to be an ideal vehicle for the dispensing of zinc salts, because it removes all possibility for zinc to form basic complexes and lowers the *p*H without excessive buffer action at this low *p*H value.

Group 2—Ophthalmic Drugs

Salts of:

Atropine	Eucatropine
Ephedrine	Homatropine
Pilocarpine	

Group 2 includes those ophthalmic drugs which we recommend be dispensed in a phosphate buffer at a *p*H 6.8. It was found that the loss in activity of these drugs after 30 days was only 10% to 20%, which was considered permissible. It was also found that when these drugs were buffered in the acid region (*p*H 2–3) they were chemically very stable; however, their physiological activity was at a minimum. In referring to Equation II it will be observed that by decreasing the hydrogen ion concentration, the concentration of free base is increased. With the increased concentration of free base the physiological activity is intensified and reaches its optimum effect at a *p*H 7 to 7.4. This effect was reported first by Gifford and Smith (1) and by Blok (19) with solutions of pilocarpine, atropine, and homatropine.

Schou, *et al.* (20) show that atropine sulfate was stable when maintained at a *p*H range between 2.8 and 6.0 and that there was distinct deterioration at *p*H 7, which was intensified as the *p*H went beyond neutrality into the alkaline range. Vincke and Oelkers (21) have pointed out that alkaline mixtures of atropine should not be dispensed because decomposition occurs in a very short time. Blok (19) reports that a 0.5% atropine solution underwent a 44% decomposition, and a solution of homatropine showed an 89% deterioration in a period of one month when dispensed in an alkaline borax-boric acid buffer (*p*H 8.3). Since the *p*H necessary for maximum stability (*p*H 2) and the *p*H for maximum activity (*p*H 7.4) do not coincide, we feel that it is justifiable to sacrifice a small portion of the intensity of therapeutic activity in order to obtain a stable pharmaceutical product.

In order to determine an optimum *p*H level where good physiological activity is obtained without sacrificing chemical stability, a systematic study of the stability of the drugs at various *p*H levels shown in Table VI was undertaken.

The actual tests in the laboratory were carried out on solutions that were heated for a period of sixty minutes at 90° to 100°. This procedure was found to simulate the hydrolysis and oxidation that occurs in eserine solutions on standing at room temperature for thirty days or more. It was therefore adopted as a standard procedure because it eliminated delays in studying these solutions.

The quantity of tropic acid formed was used as a

criterion for determinination of the decomposition for atropine, while mandelic acid determinations were used in the case of homatropine. Pilocarpine decomposition was determined by recovery with chloroform. Practical experience with ephedrine solutions indicates that it is somewhat more stable than pilocarpine.

TABLE VI.—THE STABILITY OF VARIOUS ALKALOIDAL SALTS AT DIFFERENT *p*H LEVELS

Solution	*p*H 3	*p*H 4	*p*H 5	*p*H 6	*p*H 6.8	*p*H 7.4
Atropine sulfate, 1%	S	S	S	S	S.D	D
Ephedrine sulfate, 1%	S	S	S	S	S	D
Homatropine HBr, 1%	S	S	S.D	S.D	S.D	D
Pilocarpine HCl ,1%	S	S	S	S	S	D

Key: S—Less than 10% decomposition in 30 days.
S.D—Less than 20% decomposition in 30 days.
D—Over 20% decomposition in 30 days.

A comparison was made of the physiological activity of drugs in Group 2 when dispensed in a phosphate buffer (*p*H 6.8) and in an alkaline phosphate buffer (*p*H 7.4). These tests were performed on a group of ten normal individuals. The procedure consisted of instilling a 0.5% pilocarpine solution adjusted to a *p*H 7.4 in the left eye and a 0.5% solution at *p*H 6.5 in the right eye. Neither eye showed any appreciable difference in miosis. The same was true with atropine and homatropine, which gave no difference in mydriosis on either eye. But when these drugs were instilled at *p*H 4 a definite decrease in miosis and mydriosis was observed.

BUFFER SOLUTION FOR GROUP 2 DRUGS

Sodium Acid Phosphate (NaH_2PO_4), anhydrous	4.003 Gm.
Disodium Phosphate (Na_2HPO_4), anhydrous	4.73 Gm.
Sodium Chloride, c. p.	4.30 Gm.
Alkyl dimethyl benzyl ammonium chloride	1–50,000
Distilled Water, q. s.	1000 cc.
Freezing point depression, −0.56°	*p*H 6.8

Eserine Solutions

Solutions of eserine salicylate decompose readily by hydrolysis and oxidation. The rate of decomposition varies according to temperature, *p*H, and exposure to light. According to Salway (22) the following represents the decomposition of eserine solutions:

$$\underset{\text{Eserine}}{C_{15}H_{21}O_2N_3} + HOH \rightarrow \underset{\text{Physovenine}}{C_{14}H_{18}O_2N_2} + \underset{\text{Methylamine}}{CH_3NH_2} \qquad \text{(III)}$$

$$\underset{\text{Physovenine}}{C_{14}H_{18}O_2N_2} \rightarrow \underset{\text{Eseroline}}{C_{13}H_{18}ON_2} + CO_2 \qquad \text{(IV)}$$

The oxidation products of eserine solutions unlike most other ophthalmic alkaloidal salts maintain the therapeutic activity of eserine. This has been demonstrated by Gifford and Smith (1) and Hicks (9). However, probably due to the formation of methylamine as shown by Equation III, unstabilized solutions of eserine become extremely irritating on long standing. For this reason solutions of eserine that have turned pink should not be dispensed. Krantz, Carr, and Musser (23) suggested the addition of sodium formaldehyde sulfoxalate in combination with a phosphate buffer at *p*H 6.2 as an antioxidant. Hicks (9) used the Krantz, Carr, and Musser (23) solution clinically and reported that eserine solutions, kept for six months without developing any color, were highly successful in producing miosis and in reducing tensions in cases of glaucoma.

Again according to Equation II eserine solutions having a *p*H of 6 to 7 have a relatively high concentration of free base available to the eye tissue. It has also been found that although the eserine base has strong irritating properties, if we increase the hydrogen ion concentration to a *p*H of approximately 5, much of the initial burning sensation usually associated with eserine solutions can be eliminated. Not only does the lowering of *p*H produce a less irritating eye drop, but it also increases chemical stability. It is important to remember that eserine solutions should not be strongly buffered at a *p*H 5, but should be adjusted with a weakly dissociated acid such as boric acid. When the *p*H of eserine solution is brought to *p*H 5 with boric acid, sodium formaldehyde sulfoxalate cannot be used as an antioxidant because it will decompose into formaldehyde and formic acid which will cause intense irritation.

We have found that a 0.356 *M* boric acid solution containing 0.1% sodium metabisulfite[1] is an excellent vehicle for eserine. Eserine solutions have been kept for periods of several months without developing color and were found to be completely stable. This type solution of eserine has been used clinically for four years and has been found to be very successful in producing rapid and complete miosis and for the relief of glaucoma tension. The following vehicle is recommended for the preparation of eserine solutions:

SOLUTION 3 FOR PREPARATION OF ESERINE SOLUTIONS

Boric Acid, c. p.	0.356 *M*
Sodium Metabisulfite	0.1%
Alkyl Dimethyl Benzyl Ammonium Chloride	1–50,000
Distilled Water	q. s.

Sulfa Drugs

Five per cent solutions of the sodium salts of sulfathiazole and sulfadiazine vary in alkalinity

[1] Probably a better antioxidant can be found. Experiments with sodium sulfite in boric acid solution are at present under consideration.

from *p*H 9 to 10 and the critical point before precipitation of the free sulfonic acid is around 8.2. The excess alkalinity of these solutions can be reduced to a minimum by the use of a borate buffer of very low buffer capacity. This is accomplished by the simple dilution of the Palitzsch Borate Buffer (13) at a *p*H 8.6. The following formula is recommended for dispensing the sodium salts of sulfathiazole and sulfadiazine.

SOLUTION 4 FOR PREPARATION OF SODIUM SALTS OF SULFATHIAZOLE AND SULFADIAZINE

Modified Palitzsch Borate Buffer, *p*H 8.6	200 cc.
Alkyl Dimethyl Benzyl Ammonium Chloride	1–50,000
Distilled Water, q. s.	1000 cc.

Penicillin Ophthalmic Solution

The instability of Penicillin in aqueous solutions is generally known, but beyond this there is little information available as to the stability of such solutions at different *p*H levels and at different temperatures. Foster and Wilker (24) conducted experiments on buffered solutions of penicillin at *p*H levels of 2.0, 2.6, 2.9, 4.8, 5.8, 6.8, 7.9, and 10.3. They concluded that penicillin is exceedingly labile in a solution having a *p*H below 4.8 or above 7.9, losing all activity in a matter of hours. Further experiments by Benedict, *et al.* (25) indicate that the optimum stability of penicillin in aqueous solution was between *p*H 5.6 and 6.1 and that the pure crystalline material had an optimum stability at *p*H 6.5 at 24°. We have found that penicillin may be successfully prepared for ophthalmic use in concentration of 1000 Oxford Units per cc. in a phosphate buffer of *p*H 6.5. The cylinder-plate assay of Schmidt and Moyer (26) showed no significant loss of potency at room temperature (24°) for periods of two to three days and with only 50% loss of potency at the end of ten days. When stored in the refrigerator (10°) no significant loss occurred at the end of a period of thirty days. A satisfactory penicillin ophthalmic solution may readily be prepared by the following formula:

SOLUTION 5 FOR PREPARATION OF PENICILLIN SOLUTIONS

Phosphate Buffer (Sørensen), (see Table V)	*p*H 6.47	100 cc.
Sodium Chloride		0.5 Gm.
Penicillin Sodium	100,000 Oxford Units	

SUMMARY

1. A systematic study of ophthalmic solutions was undertaken in order to evaluate the effect of the following: buffer systems, buffer capacity, hydrogen ion concentrations, osmotic effect, equilibrium concentration of the free base and chemical stability.

2. Equilibrium concentration of the free base and chemical stability are functions of the hydrogen ion concentration.

3. It was found that in many cases consideration of the equilibrium concentration of the free base and stability of the solution was more important than critical adjustment of *p*H isohydric with tears.

4. Because of the eye's ability to tolerate variation in *p*H we feel justified in advocating a reasonable modification of *p*H where chemical stability is important.

5. Critical adjustment of osmotic pressure was found to be unnecessary. Also it is felt that the lacrimated tear should not be the basis for determining the tonicity of ophthalmic solutions, but that an osmotic effect equal to physiological saline should be sought.

6. An evaluation and analysis was made of different buffer systems and their usefulness as pharmaceutical buffers.

7. A study was made to determine the optimum tonicity and *p*H value for ophthalmic solutions.

8. The most important ophthalmic drugs were classified into five groups. Five buffer systems are recommended to the pharmacist for compounding prescriptions calling for these drugs.

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The Stability of Commercial Crystalline Sodium Penicillin in Solution*

By SAMUEL MOLINAS and HENRY WELCH

The results of studies on the stability of crystalline sodium penicillin in sterile distilled water are reported.

IT HAS BEEN known for some time that crystalline penicillin, though stable in the dry state, is not stable in aqueous solution. Benedict, *et al.*, in 1945 (1) found that the optimum *p*H for the stability of crystalline sodium penicillin solution was *p*H 5.6–6.0. E. L. Smith, in 1946 (2) conducted a similar study and found that the half life of this drug at *p*H 6.0 was 14 days at a temperature of 24°. Most of the work done on the stability of penicillin in the aqueous state has been done with buffered solutions. The present studies are concerned mainly with the stability of crystalline sodium penicillin in sterile distilled water and were made to ascertain whether or not commercial crystalline sodium penicillin in solution met the labeling requirements of section 146.24 (c) (3) (iii) of the Penicillin Amendment to the Food, Drug, and Cosmetic Act. This section, requires that the manufacturer describe "The conditions under which such solutions should be stored, including a reference to their instability when stored under other conditions" . . . and to make the statement that "Sterile solutions may be kept in the refrigerator for one week without significant loss of potency." Since commercial penicillin, when packaged in vials of 200,000 units or less, must contain 85 per cent or more of the claimed potency in order to be certified, a "significant loss of potency" of penicillin in solution in these studies has been arbitrarily considered as a loss of 15 per cent within a period of seven days.

The samples of crystalline sodium penicillin used were obtained from four of the basic manufacturers, each sample representing a different lot of the commercial product. All samples were diluted with 20 cc. of sterile distilled water and then assayed by the cylinder plate method to determine the base potency. At the time of solution and whenever the solutions were assayed, the *p*H of each solution was obtained with a Beckman *p*H meter. The samples were stored in refrigerators at temperatures of 4–6° and 8–14°. The 8–14° refrigerator was chosen because it was felt that this type of refrigerator would give temperature approximating those of refrigerators found in homes and pharmacies.

Studies were made also of the stability of a highly purified crystalline sodium penicillin (95–98 per cent G) at various hydrogen ion concentrations. A buffering system consisting of $M/15$ KH_2PO_4 and $M/15$ Na_2HPO_4 was employed. These solutions were mixed in proper proportions to obtain hydrogen

* Received Dec. 16, 1946, from the Division of Penicillin Control and Immunology, Food and Drug Administration, Federal Security Agency, Washington, D. C.

Figure 1

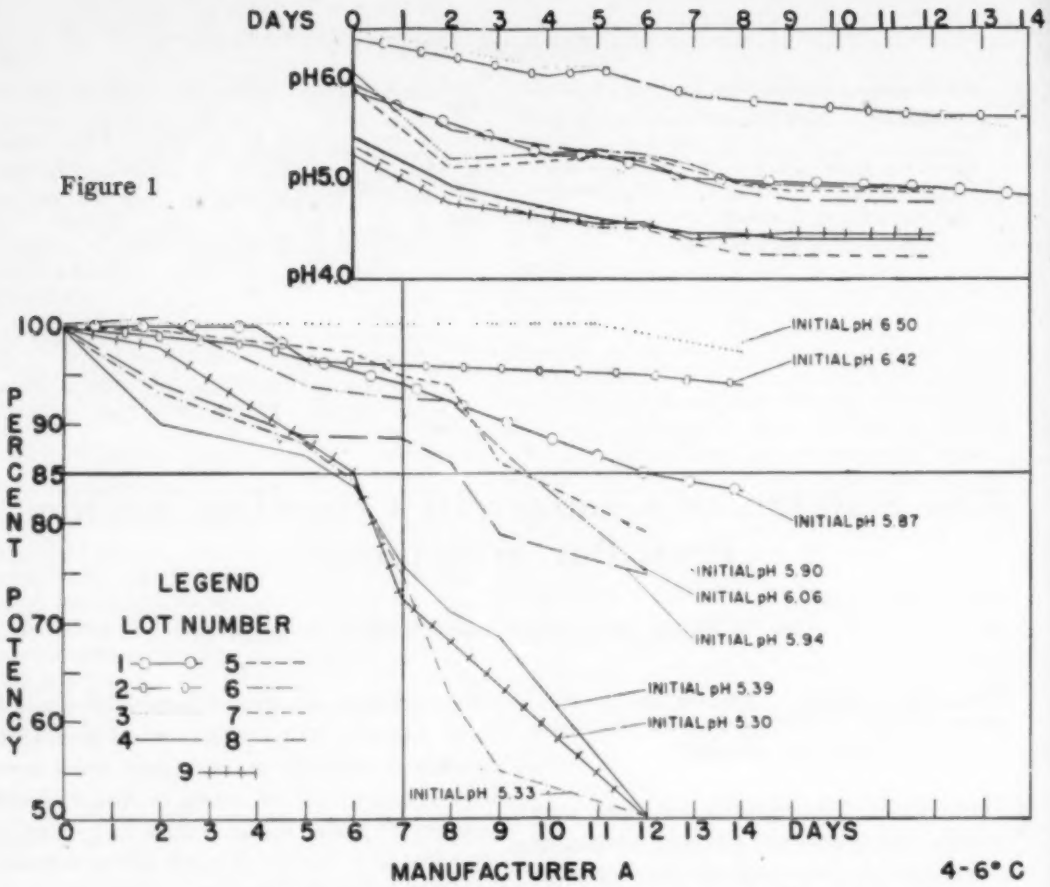
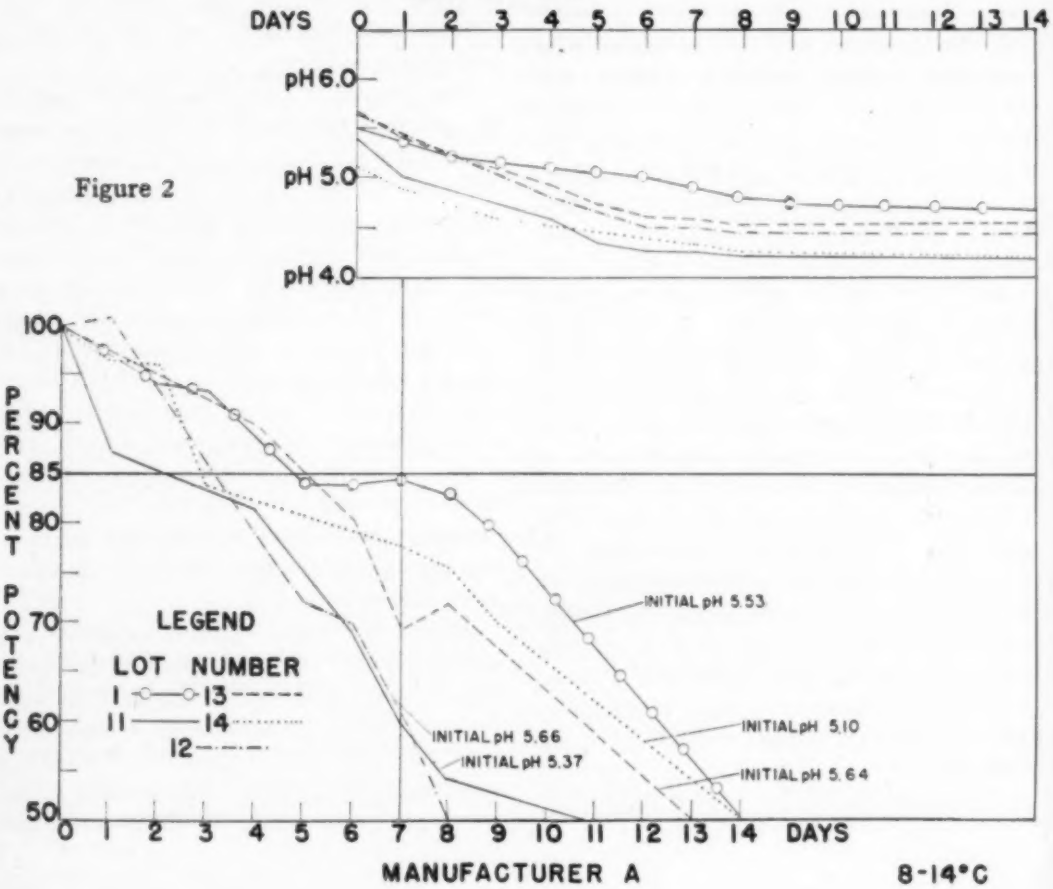


Figure 2



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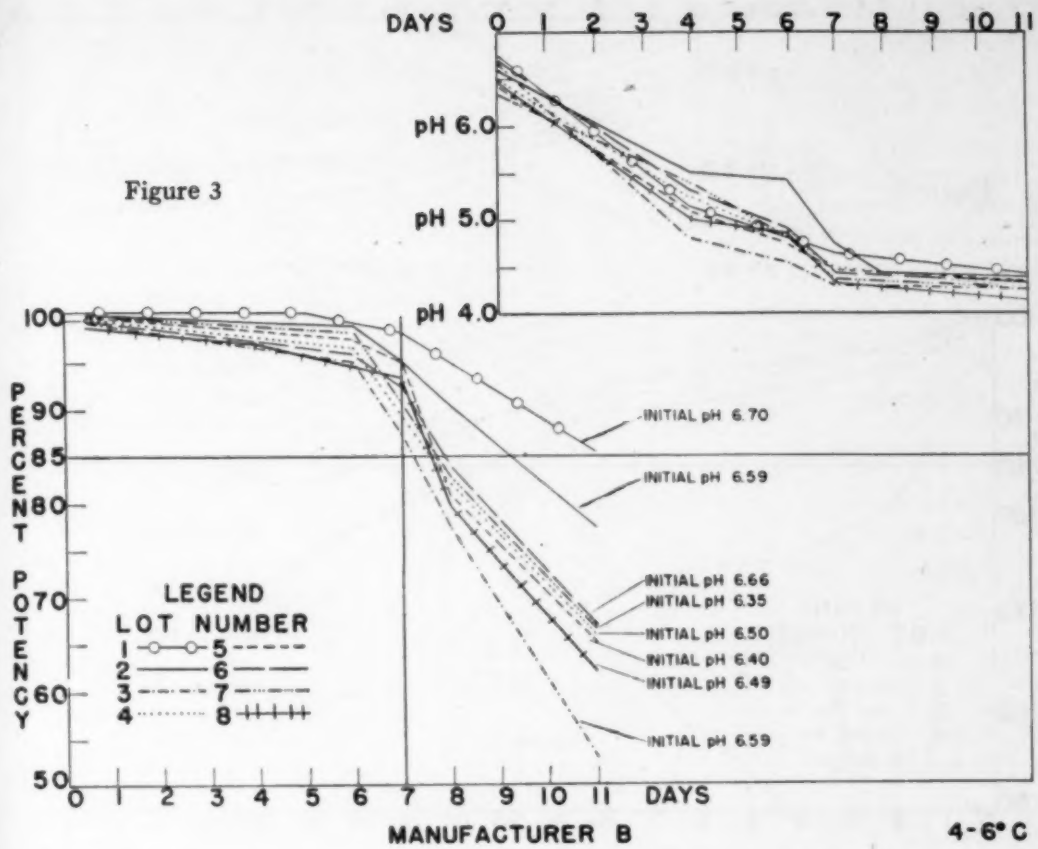

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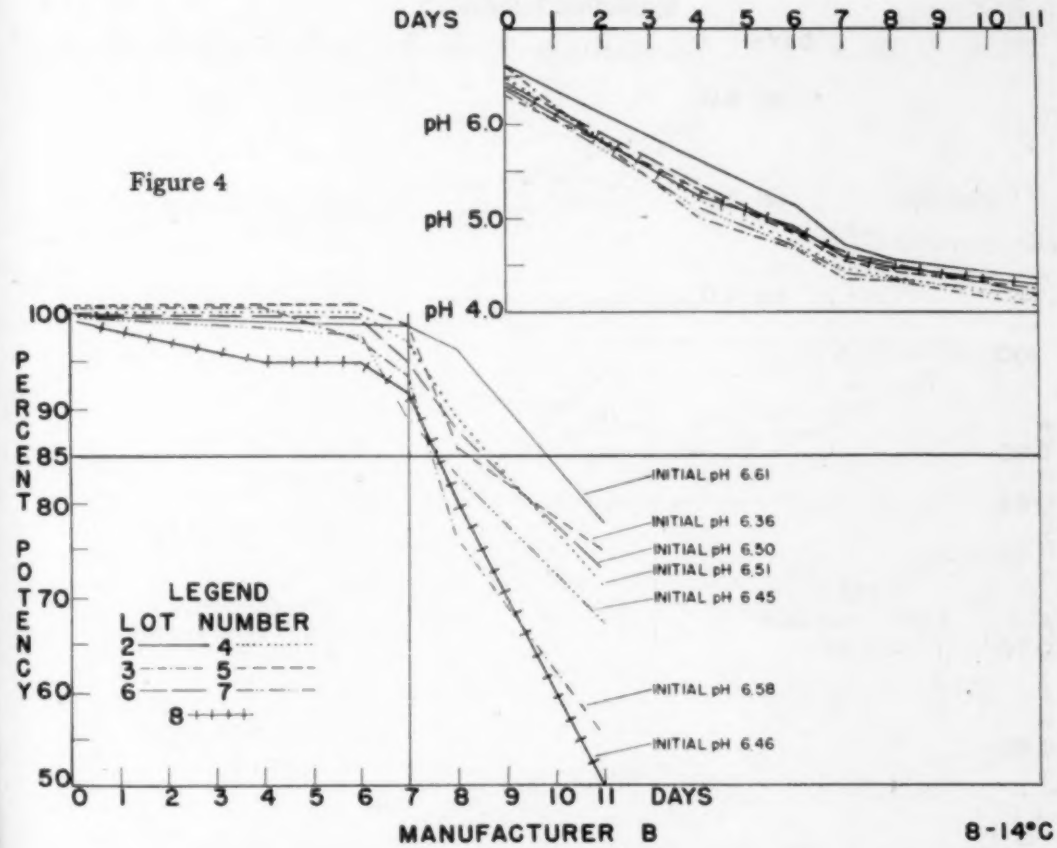


Figure 5

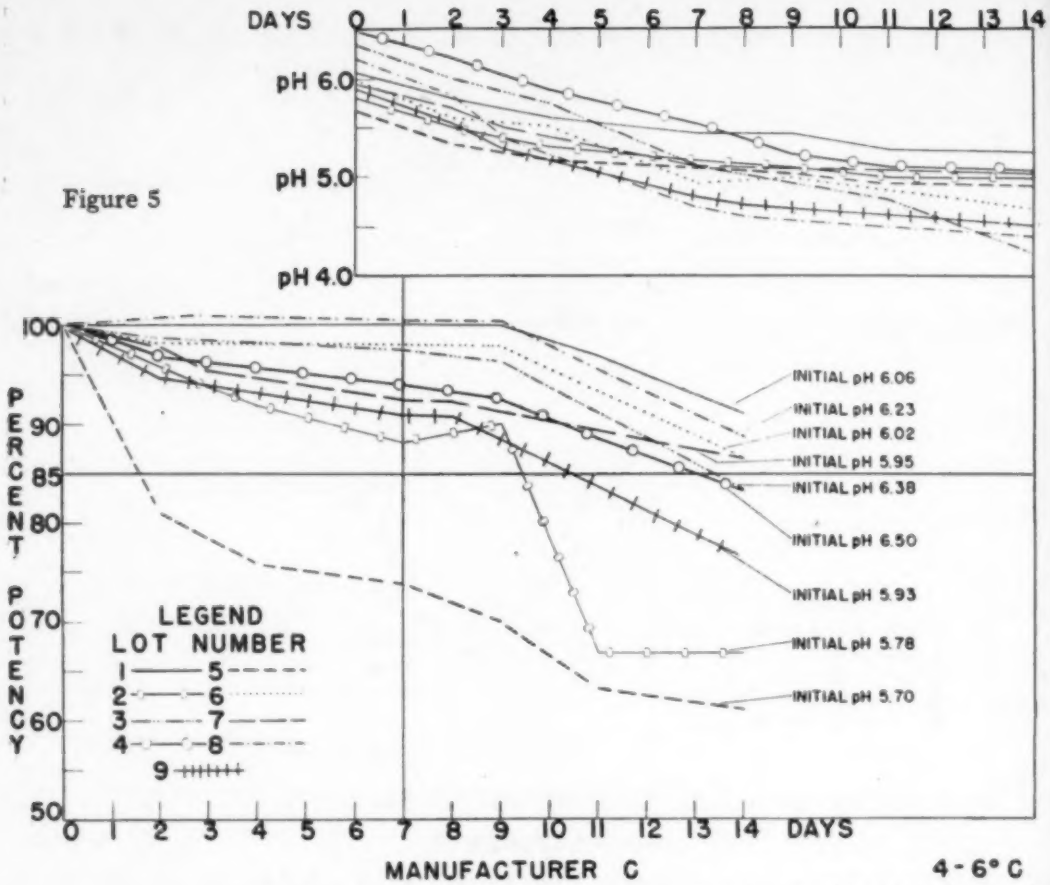


Figure 6

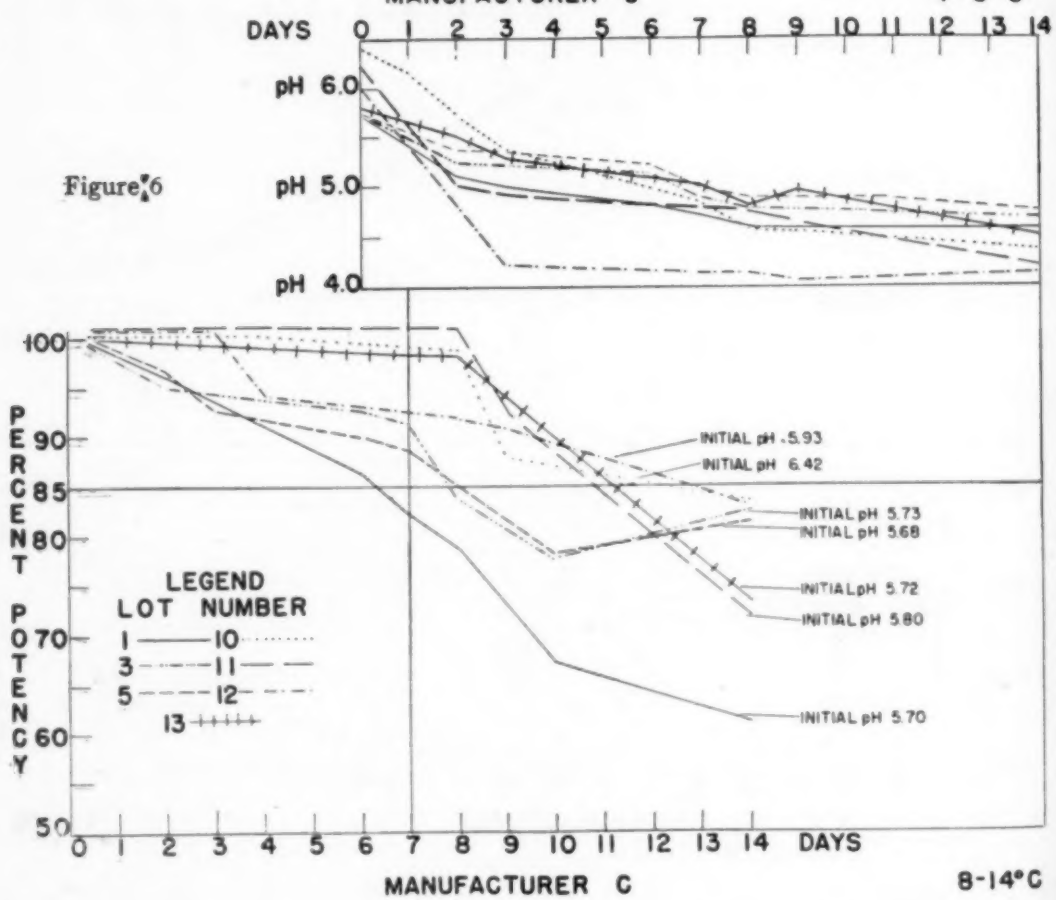


Figure 7

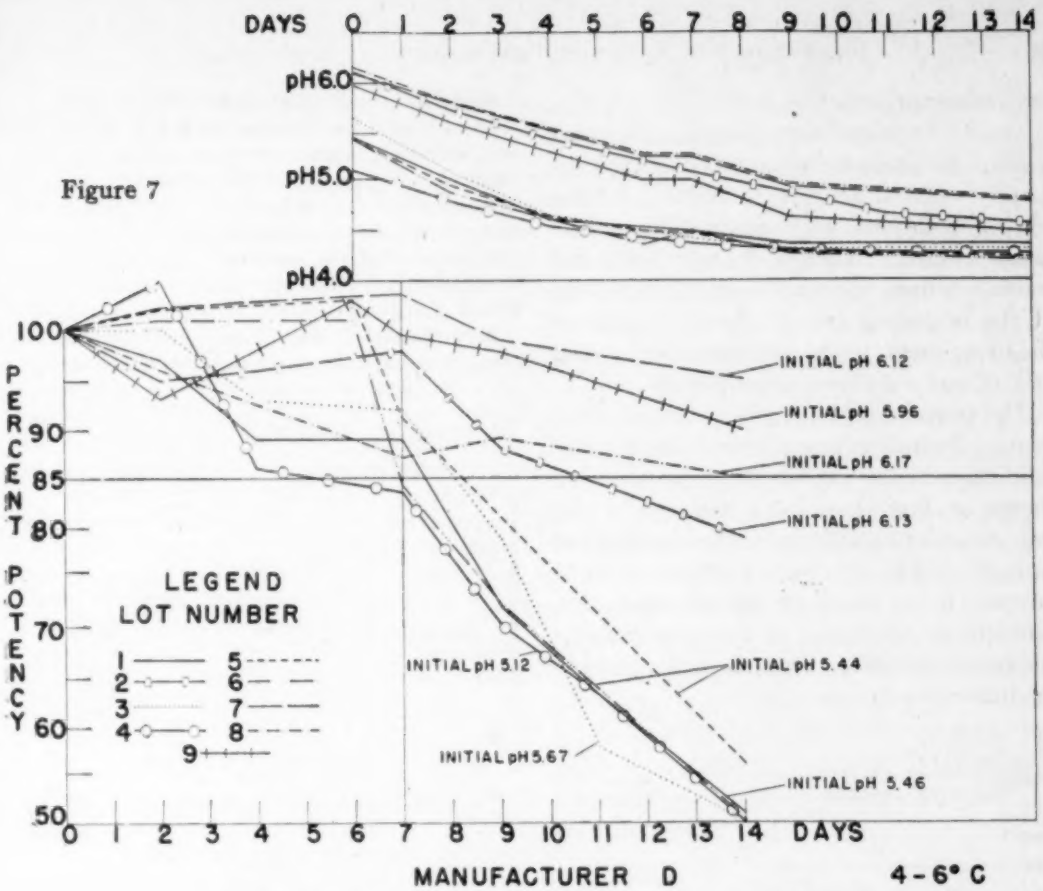
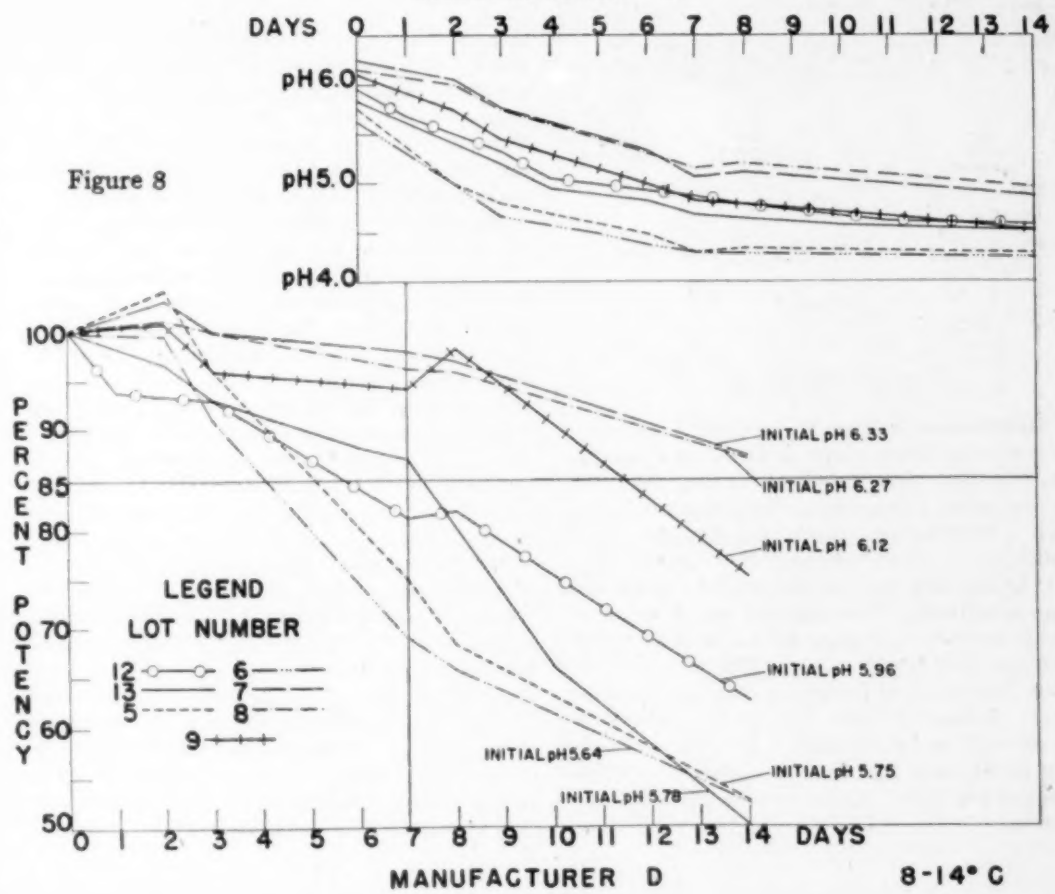


Figure 8



ion concentrations of 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5 and sufficient penicillin dissolved in them to obtain a concentration of approximately 5000 units per milliliter. Similar control solutions were made with sterile distilled water. The *p*H of the buffered and water solutions of penicillin was determined at the beginning and at the conclusion of the experiment. The solutions were stored at 4–6° and 8–14° and assayed daily.

The potency and the *p*H values of commercial crystalline penicillin sodium in water solution held for various time intervals are shown in Figs. 1–8. The samples of the four different manufacturers are designated A, B, C, and D. In Figs. 1–10, solutions of samples from the same lot of crystalline penicillin are identified by the same number and legend symbol. This also holds true for the buffered solutions.

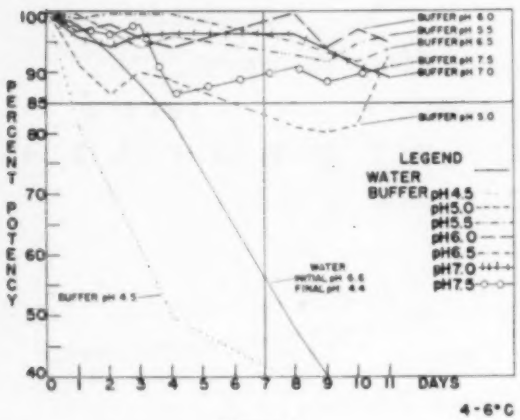


Figure 9

EXPERIMENTAL

Manufacturer A (Figs. 1–2).—Of nine lots stored at 4–6° and assayed over a period of twelve to fourteen days, three show a loss of more than 15% of their initial potency in seven days and in twelve days a 50% loss in potency (Fig. 1). The initial hydrogen ion concentrations of these solutions were 5.4, 5.3, 5.2 and the final values were 4.4, 4.4, and 4.3, respectively. The solutions which are most stable are those with initial *p*H values of 6.4 to 6.5. Five lots were tested at 8–14° (Fig. 2). All solutions show losses in potency of over 15% in seven days. It should be noted that Lot No. 1 was tested at both 4–6° and 8–14° (Figs. 1–2). The curves for this sample show that there is a greater loss in potency at the higher temperature for the same time interval.

Manufacturer B (Figs. 3–4).—The solutions of samples from 8 lots tested at both 4–6° and 8–14° are fairly stable over a seven-day period but drop rapidly after that time. The initial *p*H values of these solutions ranged from 6.4 to 6.7, but at the end of seven days they possess values of 4.3 to 4.7. The stability of the solutions stored at 4–6° and 8–14° are similar as evidenced by the curves obtained.

Manufacturer C (Figs. 5–6).—At the end of the seven days only one of the nine lots tested at 4–6°

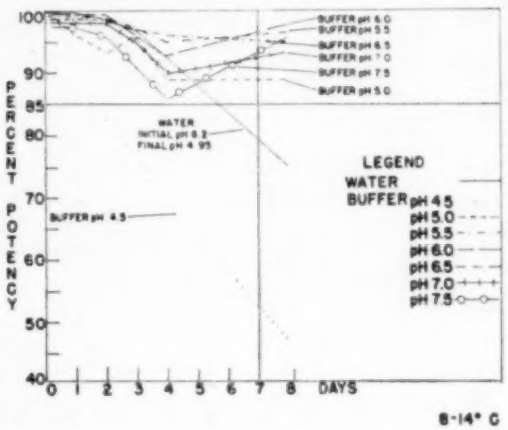


Figure 10

(Fig. 5) shows a loss of over 15% of its initial potency. Its initial *p*H was 5.8. The others, having initial *p*H values of 5.8–6.4, are relatively stable over a fourteen-day period. Three of the seven lots tested at 8–14° (Fig. 6) were tested at 4–6°. As in the case of Manufacturer A (Lot No. 1), the loss in potency at 8–14° is much greater than at 4–6°.

Manufacturer D (Figs. 7–8).—Samples of nine lots were tested at 4–6° and only two were found to have losses greater than 15% of the initial potency over a period of seven days; their initial *p*H values were 5.12 and 5.44. At the end of fourteen days, five lots having initial *p*H values from 5.12 to 5.60 show losses of 46–50% of their initial potencies, while the four lots whose *p*H values were 6.0 or higher were more stable over the same time period (Fig. 7). Of the seven lots tested at 8–14° (Fig. 8) five were tested simultaneously at 4–6° (Fig. 7). These lots were more stable at the lower temperatures.

Buffered Solutions (Figs. 9–10).—The potencies of solutions of a purified crystalline sodium pencillin buffered at *p*H 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 are stable at both 4–6° (Fig. 9) and 8–14° (Fig. 10), but solutions buffered at *p*H 4.5 are not stable and lose more than 15% of their initial potency in a very short time at both temperatures (Figs. 9–10). Solutions of this purified crystalline sodium penicillin in sterile distilled water show losses of potency greater than 15% in 3–6 days at both 4–6° and 8–14° even though their initial *p*H values were 6.6 and 6.2.

In the process of manufacture of a highly purified crystalline penicillin, penicolloic acid may be formed which causes degradation of the penicillin when it is placed in solution in distilled water. This degradation results in the production of more acid with subsequent further breakdown of penicillin. The action appears to be an autocatalytic one.

The experiments on the stability of commercial crystalline penicillin show that irrespective of *p*H practically all of the samples retain more than 85% of their initial potency for a period of three days. At the end of a seven-day period, however, only solutions having *p*H values of 6.0 to 6.7 are reasonably stable while those having a *p*H of 5.12 to 5.80 lose 15% or more of their initial potencies in this time period. It is of interest that all samples of commercial crystalline sodium penicillin having an initial *p*H of 6.0–6.5 lost less than 15% of their initial potency in seven days while the purified crystalline sodium salt in distilled water lost more than 20% of its initial potency over the same *p*H range for the same time period. These limited observations would seem to indicate that at *p*H values above 6.0 some factor in addition to the hydrogen ion concentration contributes to the increased stability of the commercial crystalline product.

SUMMARY AND CONCLUSIONS

The solubility of solutions of crystalline sodium penicillin of four of the basic manufacturers was determined at 4–6° and 8–14°. Those solutions whose initial *p*H values were 6.0–6.5 did not lose significant activity over a period of seven days while those with hydrogen ion concentrations of 5.12 to 5.80 show losses in potency in excess of 15 per cent.

Solutions of commercial crystalline sodium penicillin maintained at 4–6° are more stable than similar solutions maintained at 8–14°.

The critical *p*H for solutions of highly purified crystalline sodium penicillin was found to be *p*H 4.5 at either 4–6° or 8–14°. This was also true in general for most of the lots of commercial crystalline sodium penicillin. All penicillin tested was quite unstable at a *p*H of 4.5 or lower, and the highly purified crystalline sample in distilled water was very unstable even though the initial *p*H values were from 6.2 to 6.6.

The optimum *p*H for maintenance of the activity of commercial crystalline sodium penicillin in solution appears to be *p*H 6.0–6.5. It appears that as the purity of the crystalline sodium penicillin is increased, the product becomes more unstable in water solution.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Erythritol
Erythritol anhydride
Glutaconic acid
l-Glyceric acid
l-Glyceraldehyde
Thioveronal
8-Quinolinesulfonic acid
2,3,4,5-Tetramethylhexane
Polyphenol oxidase
Laccase
Adenine dinucleotide
9-α-Urobilin
Agmatine sulfate
9-Amylanthracene
Glucurone
l (+)-Glutamine
Tropic acid
d-Fucose
Agaric acid
d-Quercitol

Quaternary Ammonium Compounds as Preservatives*

By L. F. TICE and A. W. MOORE

The antiseptics employed were cetyl pyridinium chloride (Ceepryn) and *p*-tertiary octylphenoxyethoxyethyl dimethyl benzyl ammonium chloride (Phemerol). Very low concentrations of these antiseptics were found effective in preserving both gelatin and sucrose solutions. Minimum effective concentrations of each antiseptic and several inconsistencies in results are reported.

THE SELECTION of a suitable preservative is a frequent problem in the formulation of pharmaceuticals and, like the "ideal" antiseptic, an ideal preservative has not yet been found. The unusual bactericidal efficiency of certain quaternary ammonium compounds has received considerable attention and a number of papers on this subject have appeared since the original work by Jacobs and his co-workers (1–3) in 1916. Thus Shelton, *et al.* (4,5) and Quisno and Foter (6) reported on the germicidal action of certain compounds derived from cyclic amines and found cetylpyridinium chloride (Ceepryn) to be the best tested.

"Phemerol" (*p*-tertiary octylphenoxyethoxyethyl dimethyl benzyl ammonium chloride) and "Zephiran Chloride" (a mixture of alkyl dimethyl benzyl ammonium chlorides) have also been reported as highly effective cationic antiseptics (7, 8). Cetylpyridinium chloride has been shown to have remarkably high bactericidal efficiency as determined by a measurement of its critical killing dilution (9). By this method of testing it was found to kill *Staphylococcus aureus* in a dilution of 1:65,000 and even killed it in 10 per cent serum in a 1:11,500 dilution.

The toxicity of cetyl pyridinium chloride has been investigated by Warren, *et al.* (10) who studied its acute and chronic toxicity. They found that oral administration of this compound in an amount of 400 mg./Kg. killed only one out of six rabbits and that in chronic toxicity tests amounts of 10 and 100 mg./Kg. daily for four weeks had no significant harmful effects, the majority of test animals gaining in weight during the test period.

These reports of high bactericidal efficiency coupled with low toxicity caused the authors to consider the possible application of these compounds as preservatives. With this object in mind the following experiments were conducted.

EXPERIMENTAL

Since both Ceepryn and Phemerol were available in pure form, they were selected for this study. Gelatin represents an unusual substance in that it is subject to fungous growth at low *p*H values and to bacterial growth at higher *p*H values. Consequently, an acid type gelatin (Pharmagel A) and a more nearly neutral gelatin (Pharmagel B) were selected for this study. Pharmagel A (1%) was found to have a *p*H of 4.2 and Pharmagel B (1%) a *p*H of 5.9. By using gelatins at these different *p*H levels both bacteriostatic as well as fungistatic action of the antiseptics could be determined.

It has been well established that at the *p*H level of the Pharmagel A most bacteria are inhibited and only fungi grow readily, while at that *p*H level found in Pharmagel B bacteria thrive. Testing the antiseptics as preservatives at different *p*H levels was also considered advisable in view of the reported variations in bactericidal efficiency that have been observed at different *p*H levels (6, 9) using the cationic antiseptics.

In order to provide a rigid test a 2% solution of Pharmagel A and a 2% solution of Pharmagel B were exposed in Petri dishes for four days. Each became heavily contaminated with organisms. Four parts of water were then added and a uniform suspension made.

This suspension of organisms was then used as an inoculum in all tests on each gelatin, 0.1 cc. of the suspension being added per 100 cc. of gelatin solution. Various concentrations of Pharmagel A, Pharmagel B, and sucrose were prepared, as well as mixtures of gelatin and sucrose. The minimum amount of Ceepryn and Phemerol necessary to preserve these solutions was then determined after inoculating the sample as already described. The last reading was made after approximately eight months' storage at room temperature (20–30°). The results are shown in Table I.

* Received Sept. 5, 1946, from the Department of Pharmacy, Philadelphia College of Pharmacy and Science.
Presented to the Scientific Section of the A. PH. A., Pittsburgh meeting, August, 1946.

DISCUSSION

In examining the samples at regular periods the same inconsistencies were observed as have been reported in testing the germicidal activity of the quaternary ammonium antiseptics (11, 12). For example, in a series of tubes with increasing amounts of the antiseptic several negative tubes might be followed by one containing more antiseptic but showing growth. In such cases the concentration beyond which no growth was observed was taken as the minimum effective concentration. Those samples containing combinations of gelatin and sucrose behaved in an anomalous manner. In the first series prepared, 0.1% of Ceepryn and Phemerol proved ineffective. Since this was the highest concentration employed in this series, a second was prepared. In this series concentrations considerably below 0.1% proved effective.

TABLE I.—EFFECTIVE CONCENTRATION OF CEEPRYN AND PHEMEROL IN PRESERVATION OF GELATIN, SUCROSE, AND MIXTURES OF GELATIN AND SUCROSE

Pharmagel A, %	Pharmagel B, %	Sucrose, %	Minimum Effective Concentration Ceepryn, %	Minimum Effective Concentration Phemerol, %
1	..	..	0.003	0.025
..	1	..	0.005	0.01
5	..	..	0.005	0.025
..	5	..	0.008	0.02
10	..	..	0.045	0.1
..	10	..	0.1	>0.1
..	..	10	0.0005	0.005
..	..	25	0.0005	0.003
10	..	10	0.1	>0.1 and 0.04[a]
..	10	10	>0.1 and 0.04[a]	>0.1 and 0.06[a]

[a] Results of duplicate experiment (see Discussion)

Coacervation was observed in many of the samples containing Pharmagel B plus the cationic antiseptic. This phenomenon of coacervation between a negatively charged gelatin and the cationic quaternary ammonium compounds has been reported previously (12). Although higher concentrations of the antiseptic were required to preserve the negatively charged gelatin (Pharmagel B), it is not believed that this is due to inactivation of the antiseptic through coacervation but rather due to the fact that more resistant organisms grow in gelatin at the higher *p*H level of this gelatin. There seems to be some disagreement on whether coacervation with gelatin inactivates the cationic antiseptic but Tice and Pressman (12) were unable to demonstrate any reduction in bactericidal efficiency in the presence of a negative gelatin.

In the case of the sucrose solutions a slight sediment appeared which seemed to increase with increasing amounts of the antiseptic. Microscopic examination failed to show any organized growth.

In all the experiments performed Ceepryn seemed to be considerably superior to Phemerol as a preservative since lower concentrations consistently were required under identical conditions of testing.

With both substances, however, there seem to be possibilities of use based on their preservative action. Liquid products for oral use would need some flavor to mask the rather unpleasant taste of these compounds. Solid dosage forms, such as capsules, and external products would not give evidence of its presence.

SUMMARY

1. The preservative action of cetyl pyridinium chloride (Ceepryn) and *p*-tertiary octylphenoxyethoxyethyl dimethylbenzyl ammonium chloride (Phemerol) on gelatins, sucrose, and mixtures of gelatin and sucrose has been investigated.

2. The minimum effective concentrations of each antiseptic for different concentrations of gelatin and sucrose are reported.

3. Numerous inconsistencies in results are described.

4. Although both cationic antiseptics studied showed value, Ceepryn gave consistently better results.

5. The use of such substances as preservatives warrants further consideration.

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A Contribution to the Pharmacology of Succinic Acid and Its Sodium and Magnesium Salts*,†

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The results of a study on the effects of succinic acid, sodium succinate, and magnesium succinate in cats are reported. Large doses of these compounds produce numerous acute disturbances, the causes of which appear to be nonspecific. The report indicates that the succinate ion is substantially inert and that it exerts no specific toxic effects.

A FAIRLY extensive literature has accumulated in recent years concerning the role of succinic acid in metabolism and its relation to oxidations and enzyme functions. These investigations have provided the basis for several possible therapeutic applications of succinates which are still in the controversial stage, such as its use in the treatment of diabetic acidosis (1), as an antidote to barbiturate poisoning (2–7), protection against poisoning by dithiols (8), and its use in the place of salicylates in the treatment of rheumatic fever (9).

Aside from its metabolic behavior and relation to enzyme systems, there is relatively little known concerning its pharmacology and toxicology. Soskin and Taubenhaus (2) found sodium succinate nontoxic in rats in intramuscular doses of 6 Gm. per Kg. in 20 per cent solution. There was considerable absorption as judged by the deposits of glycogen up to 7 per cent in the liver seen twenty-four hours later. There were no symptoms of poisoning and sections of the liver and kidneys showed no injury. Gubner and Szucs (9), who gave the calcium double salt of benzoic acid and the benzyl ester of succinic acid to 55 patients with rheumatic fever in an average dose of 4.2 Gm. daily (representing about 2 Gm. of succinic acid daily) for an average of twenty-seven days, observed no toxic effects except flushing and slight mental confusion in one who received 8 Gm. daily. Rose (10) found doses of approximately 1.5 to 3 Gm. of sodium succinate per Kg. given daily for two and three days subcutaneously in 10 per cent solution, free of toxic action on the kidney of two rabbits as shown by the blood metabolites and phenolsulfonphthalein excretion. Harding and Nicholson (11), on the contrary, concluded from experiments with five rabbits that succinic acid is a mild renal irritant, on the basis of the appearance of albumin and casts in the urine, and minimal histological changes in the kidney, after sodium succinate in doses of about 2 Gm. per rabbit (weight not stated) by intramuscular injection of an approximately 16 per cent solution. Soskin and Taubenhaus (2) mentioned no toxic effects after 22 Gm. (0.44 Gm. per Kg.) of sodium succinate by intravenous administration of a 10 per cent solution injected during a period of five hours in a patient poisoned with barbiturates and 1.083 Gm. of picrotoxin.

Active absorption of sodium succinate in man is seen from the experiments of Becker-Freyseng and Liebich (12) who observed marked alkalinization (rise of CO_2 alveolar tension, rise of urinary *p*H, fall of urinary titratable acidity, fall of urinary ammonia nitrogen) after about 23 Gm. orally three times daily for two days. There were no toxic symptoms. There are several reports which indicate that succinate is absorbed after oral administration in animals (13, 14).

The pharmacology and toxicology of several carboxylic acids, gluconic, fumaric, and hydroxyacetic, were the subjects of previous investigations in this laboratory (15–18). The present study was planned to investigate the toxicology of succinic acid in acute and chronic experiments in cats.

Succinic acid is a dicarboxylic acid with the formula:

$$COOH—CH_2—CH_2—COOH$$

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The sample of acid we used had the following physical and chemical constants: molecular weight 118.09; melting point 189–190°; boiling point 235°; specific gravity 1.562 at 15°; solubility in water 6.8 Gm. per 100 at 20° and 121 Gm. at 100°. It was administered in the form of the acid, and the sodium and magnesium salts.

ACUTE EXPERIMENTS

Preliminary experiments gave indication that the toxicity of the succinates is low and that hypertonicity might complicate the evidence of specific toxic effects. Accordingly, the concentration of sodium succinate having the same tonicity as 0.85% sodium chloride was determined. The red cell volume method was employed. To each of several special graduated tubes containing 5 cc. of 0.85% solution of sodium chloride, or varying concentrations of sodium succinate, 0.5 cc. of oxalated cat's blood was added. They were allowed to stand for one hour and centrifuged for one hour. The tube showing the same hematocrit reading without hemolysis as the sodium chloride solution represented a 2.26% solution of sodium succinate. Such a solution may, therefore, be taken as approximately isotonic with blood or equitonic with 0.85% sodium chloride.

Effect of Intravenous (Single Dose) Sodium Succinate in Cats.—The compound was administered in a 10% solution (4.4 times isotonicity) intravenously to each of 14 cats in doses varying from 0.5 to 2.5 Gm. per Kg. The essential results are summarized in Table I. Vomiting occurred after all the doses, diarrhea after doses of 1 Gm. or more. The larger doses caused weakness or prostration, and a dose of 2 Gm. or more was sometimes fatal.

TABLE I.—EFFECT OF INTRAVENOUS SODIUM SUCCINATE IN CATS (10% SOLUTION)

No. of Cats	Dose, Gm./Kg.	Effects
2	0.5	Vomiting in 3 to 4 min.; no other effects
4	1.0	Vomiting; diarrhea; ataxia
4	1.5	Vomiting; diarrhea; ataxia; weakness; tremors in one case
2	2.0	Vomiting; diarrhea; prostration; one died in 22 hr.
1	2.18	Signs of nausea; ataxia
1	2.5	Diarrhea; died in 21 hr.

Effect of Oral (Single Dose) Sodium Succinate in Cats.—In view of the fact that cats sometimes survived doses as high as 2 Gm. per Kg. intravenously, larger doses were tested by oral administration. The results of experiments in 5 cats are summarized in Table II. A dose of 5 Gm. per Kg. in a 15% solution given by stomach tube, caused prompt vomiting with no other effects. An attempt was made to prevent vomiting by the use of 1 mg. of morphine sulfate per Kg. intravenously. It was only partially successful, but the impairment of the vomiting mechanism allowed the material to pass into the bowel giving rise to a diarrhea. One animal which received 5 Gm. per Kg. together with the morphine died in five hours with camphor-like myoclonic convulsions; the respiration ceased before the heart beat. One animal received a solution of sodium bicarbonate of approximately similar tonicity and in a dose which represented an approximately similar amount of sodium, in order to ascertain the possible role of hypertonicity and/or sodium in the toxic effects of the sodium succinate. The sodium bicarbonate produced effects indistinguishable from those of the sodium succinate.

TABLE II.—EFFECT OF ORAL SODIUM SUCCINATE IN CATS

Cat No.	Dose, Gm./Kg.	Concentration, %	Effects
4	1.0	1.57	None
1	5.0	15	Vomited in 4 min.; repeated several times; no other effects
2	5.0	15	Vomited in 12 min.; repeated several times; no other effects
3	5.0	15	Received 1 mg. per Kg. morphine sulfate intravenously, 11 min. before succinate; nausea and retching but no vomiting; violent diarrhea; tremors in 5 hr.; weakness; thirst; recovered
5	5.0	15	Received morphine as above; vomiting; diarrhea; convulsions in 3 hr.; death in 5 hr. with cessation of respiration before heart beat; post-mortem—severe irritation of mucosa of stomach and small intestine
6	5.2[a]	10	Received morphine as above; nausea; no vomiting; diarrhea; convulsions in 1 hr.; death in 2 hr. with cessation of respiration before heart beat; post-mortem—same as cat 5

[a] Sodium bicarbonate.

Effect on Blood Pressure in Cats.—This was tested in each of 3 cats, anesthetized with "Dial," 0.5 cc. per Kg. intraperitoneally. The blood pressure was recorded by a membrane manometer from the carotid artery and respiration by means of a pleural cannula. The drug was given intravenously. In one cat a 2% solution (slightly hypotonic) of sodium succinate was without effect on the blood pressure, in a dose of 250 mg. per Kg., and a second dose of 500 mg. per Kg. injected slowly, over a period

of about two minutes. The second cat received a 5% solution of sodium succinate[1] (2.2 times isotonic), in single doses ranging from 100 to 500 mg. per Kg. A 500-mg. dose of the hypertonic solution even when injected fairly slowly produced vomiting and a moderate fall of the blood pressure with prompt recovery; a similar dose injected rapidly produced an abrupt and marked fall of the blood pressure, from 200 to 125 mm. systolic, also with fairly prompt recovery. The rapid recovery from the effects of these injections was shown by the fact that, within a few minutes after a total of 2.1 Gm. per Kg. given in fractions over a period of fifty minutes, the blood pressure was at approximately the level of the control. Thirty minutes later another single dose of 1.1 Gm. per Kg. produced prompt decline of the pressure to shock levels with cessation of respiration while the heart was still beating, and death. The third cat received a 15% solution of sodium succinate (6.6 times isotonic). The results were substantially similar to those of the previous cat pointing to the role of hypertonicity and speed of injection in the blood pressure effects. A total dose of 2.75 Gm. per Kg. given over a period of fifty-seven minutes proved fatal. A fairly small dose of this hypertonic solution, 250 mg. per Kg., produced vomiting, and a prompt decline of the blood pressure, from 175 to 125 mm. with prompt recovery. As noted above, twice this dose in a lower concentration more slowly injected produced no effect on the blood pressure. A comparison of the effects of 500-mg. and 750-mg. doses injected in a period of about ten seconds, with those of similar doses injected in a period of about 2 minutes, again showed the marked effect of speed of injection on the degree of blood pressure decline.

In order to secure information concerning the role of hypertonicity on the toxic effects of sodium succinate, a cat was prepared in a manner similar to that of the foregoing animals, and received intravenous injections of a solution of sodium chloride, approximately similar to those of sodium succinate with respect to tonicity (5.7 times isotonic), volume of injection, and weight of the salt. The results in these 2 comparable experiments are shown in Fig. 1. The effects of the sodium chloride solution were indistinguishable from those of sodium succinate. The last dose of the succinate, however, proved fatal, while that of the sodium chloride was not. Since the dose of sodium chloride was nearly 3 times as large on a molecular basis, the results indicate that the molecular toxicity of sodium succinate is higher than that of sodium chloride. The question arose whether the toxicity might be due to the base content of the two materials, but with respect to that point, the dose of sodium chloride which appeared less toxic contained 30% more sodium than the dose of sodium succinate. It still remains a possibility that the toxicity may not be specific for the succinate, and that the base may account for the toxicity, since the sodium is more firmly bound in sodium chloride, but more readily liberated in sodium succinate.

[1] Administered as a 5% solution of succinic acid (pH 2.3) neutralized (litmus) with sodium bicarbonate.

CHRONIC EXPERIMENTS

Chronic experiments were carried out in 8 cats, two with each of the following: succinic acid orally; sodium succinate orally; sodium succinate intravenously; and magnesium succinate orally. For control, 5 cats were studied without drugs during a similar period, 2 received sodium chloride orally, and 1 sodium acetate orally. Observations were made on the general behavior of the animals, their weight, liver function (bromsulphalein), urine (albumin, casts, and cells), blood NPN and creatinine, kidney function (P. S. P.), red blood cell count, and hemoglobin. Values for the foregoing were obtained before the drug, during the period of its administration, and in some cases after it was discontinued. Most determinations were made at weekly intervals. The samples of blood for tests were taken from the jugular vein of the cats in a fasting state. The animals were kept on a constant daily diet of 50 cc. milk, 200–250 Gm. fresh beef lung, and water without restriction.

The oral succinates were given daily (six days a week) for eleven weeks. The dose was 0.5 Gm. per Kg. in a capsule, administered on an empty stomach followed by 50 cc. of milk. The sodium chloride and sodium acetate were similarly administered in a similar dose for a period of four weeks. In the case of intravenous sodium succinate, a 10% solution was used, and was given in a dose of 0.25 Gm. per Kg. twice daily to one cat, and 0.5 Gm. per Kg. twice daily to another for four weeks.

General.—There were no signs of systemic toxicity in the general behavior of the cats throughout the period of treatment.

Weight.—The 8 cats receiving succinates were weighed weekly for from ten to seventeen weeks. The average weight before the drug was 2.89 Kg. (1.98–3.71), and the end of the treatment period, 2.98 Kg. (1.83–3.80).

Urine.—There were 52 specimens of urine (expressed from the bladder) from the cats which received oral succinates, and from 2 control cats. These were negative for albumin, casts, and cells, before and during the treatment.

Phenolsulfonphthalein Test.—This was carried out in 2 control animals, and in the 6 which received oral succinates. There were in all 52 tests, 3 weekly tests before the drug and again weekly during the first four weeks of treatment. A dose of 6 mg. of the dye was injected intramuscularly after the bladder was emptied by expression of the urine. The urine similarly obtained was tested at the end of one hour and two hours. The total percentage excretion in two hours, averaged for the 6 cats, was 51%, 55%, and 48% in the three control weeks, respectively, and 46%, 50%, 51%, and 49%, respectively, in the first four weeks of treatment. The 10 values in the 2 control animals averaged 51%.

Bromsulphalein Test.—The method of Rosenthal and White (19) was used. The 2-mg. dose of the dye was injected intravenously and the blood was tested after thirty minutes. There were in all 38

tests, in 2 control cats, in 2 cats receiving intravenous sodium succinate, and in 6 cats given the oral succinates. In the oral-treated animals, the test was made before the drug and at weekly intervals in the first four weeks, and in the intravenous-treated animals, the test was made in the third week of treatment. All samples were negative for the dye in thirty minutes after the injection.

Red Blood Cell Count.—Samples of blood were obtained from the marginal ear vein for this purpose. There were in all 108 red blood cell counts made in 4 control cats and in 6 cats which received the oral succinates. There were 3 weekly counts before the drug, and 10 weekly counts during the period of treatment. There were 6 weekly counts in the 4 cats which received no drug. There were marked individual variations between counts in the same animal (6.5 to 10.2 million per mm.^{3}) and between the average counts for different animals. There were in all 42 control counts which averaged 8.6 million per mm.^{3} as compared with an average of 11.3 million per mm.^{3} for the 66 counts during the period of treatment. The results are summarized in Fig. 2. They indicate a rise in the red blood cell count during the period of treatment with the succinates.

Hemoglobin.—This was determined by the acid-hematin method using the Sahli-Hellige hemoglobinometer. There were in all 45 values, 21 of which were controls and 24 during treatment. Determinations were made at weekly intervals for three weeks in 1 control cat, and for three weeks prior to the treatment period in 6 cats which received the

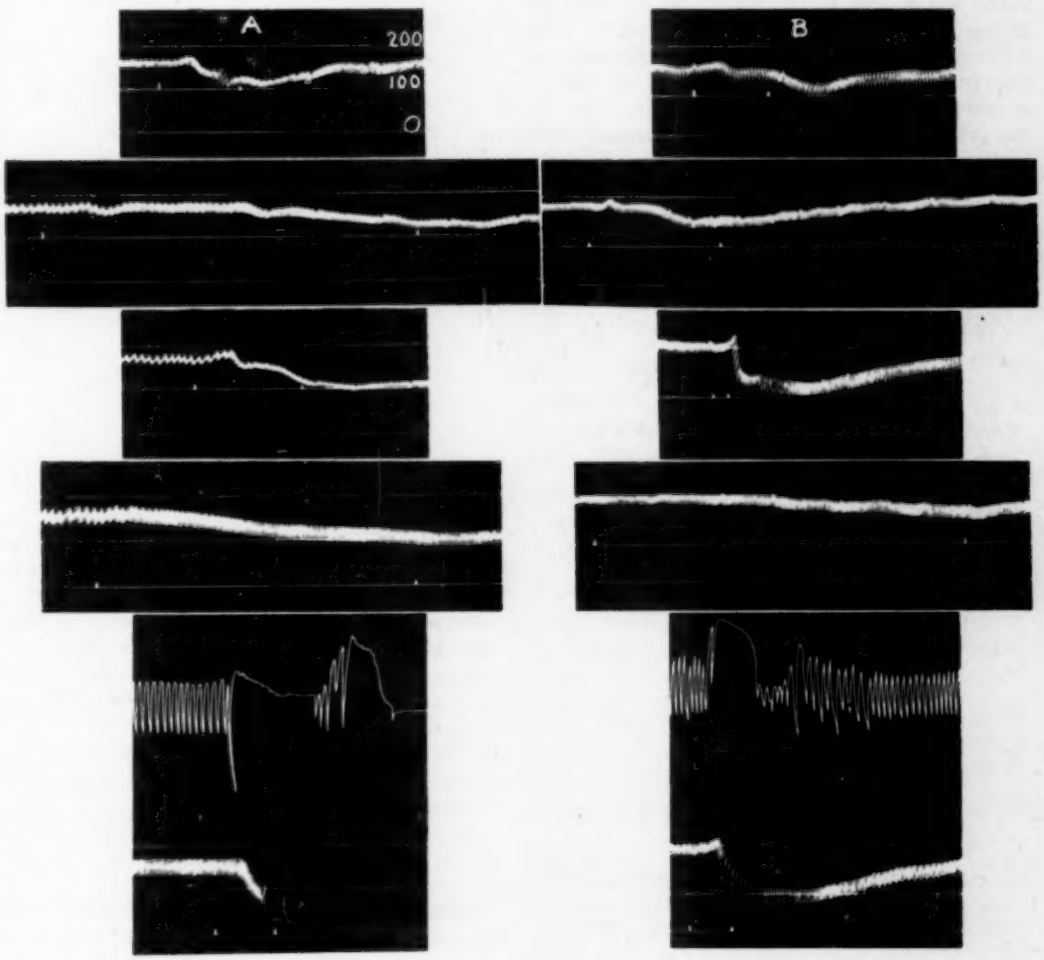

Fig. 1.—Comparison of sodium succinate and sodium chloride on the blood pressure of cats. Lines represent 0, 100, 200 mm. mercury. Series A of cat receiving 15% solution sodium succinate; series B of cat receiving 4.9% solution sodium chloride. The doses were given intravenously and were approximately comparable with respect to tonicity, intervals, speed of injection, volume of fluid, and weight of the salt. On molecular basis, each dose of sodium chloride was approximately 2.8 times that of sodium succinate. Each tracing represents the effect of a dose with the following intervals between doses: 11, 22, 16, and 8 min. Doses (reading from above down) were as follows: sodium succinate—250 mg./Kg., 500, 500, 750, 750; sodium chloride—230 mg./Kg., 460, 460, 690, 690. Intervals between white points indicate duration of injection. Note essential similarity in the effects of sodium succinate and sodium chloride, except for last dose (tracing showing blood pressure and respiration) which proved fatal in the case of sodium succinate.

oral succinates. There were 4 weekly values during the period of treatment. The values at different times in the same cats and in different cats varied greatly. The results are summarized in Fig. 2. They show a rise in the hemoglobin during the period of treatment with oral succinates, corresponding in the first three weeks to the rise in the red blood cell count.

Blood NPN.—The method of Folin and Wu was used. There were in all 162 determinations in 5 control cats and in the 6 cats treated with oral succinates. Values were obtained at weekly intervals before, during, and after the period of treatment in the case of the oral succinates. The results are summarized in Fig. 2. There was a marked rise in the blood NPN during the period of succinate treatment. The average blood NPN for 63 control values was 51.3 (S. E. 1.1) mg.%, and for 66 values during treatment, 63.3 (S. E. 1.6) mg.%. The difference of the averages is highly significant, since the ratio $(x^1 - x^2)/(\sqrt{\sigma_1^2 + \sigma_2^2})$ is 6.2, the value 3 or more being considered significant (20). The rise was present after the first week, it continued with fairly wide fluctuations, and declined to the range of the control values when the drugs were discontinued.

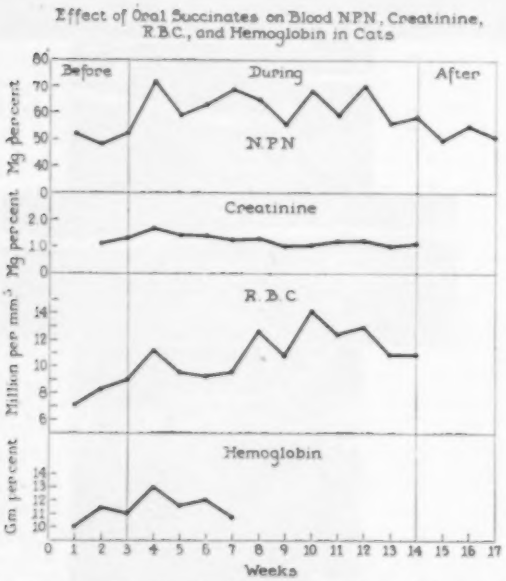

Fig. 2.—Points, during treatment periods and after, are averages of 6 cats; in control before treatment, points represent averages of 11 cats for NPN, 10 for creatinine, 10 for R. B. C., and 7 for hemoglobin.

Similar determinations, made in the case of the intravenous sodium succinates, show a similar rise in the blood NPN (Fig. 3).

Similar determinations made in the case of each of 2 cats receiving sodium chloride and 1 cat receiving sodium acetate are shown in Fig. 4. There is no significant trend.

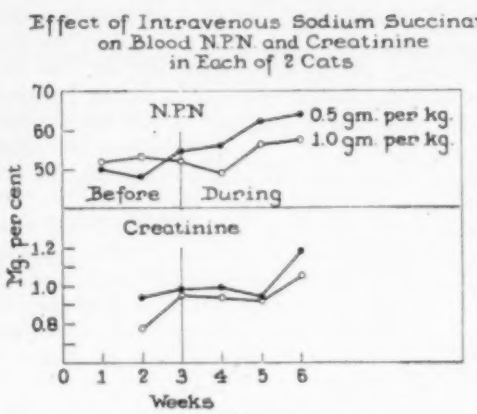

Figure 3

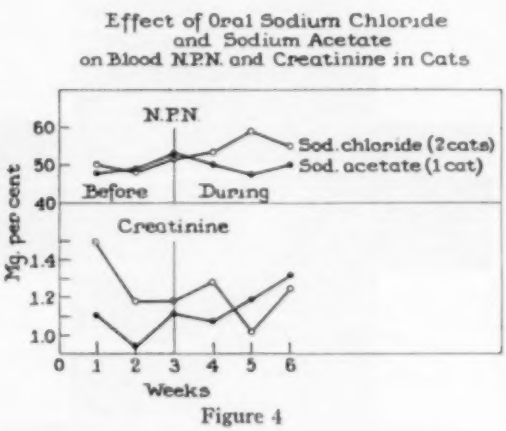

Figure 4

Blood Creatinine.—The usual alkaline-picrate method was employed. There were in all 144 determinations made at weekly intervals. The results in the case of the oral succinates are summarized in Fig. 2. There appears to be a temporary rise in the blood creatinine in the early period of treatment with oral succinates corresponding to the rise in the blood NPN.

The effect of the intravenous sodium succinate on the blood creatinine is shown in Fig. 3. There is no significant trend.

The effect of sodium chloride and sodium acetate on the blood creatinine is shown in Fig. 4. There is no significant trend.

Kidney Sections.—In view of the continued azotemia occurring during the protracted use of the succinates, the kidneys were examined histologically at the end of the experiment in the case of the 2 cats which received the daily intravenous dose of 0.5 Gm. and 1.0 Gm. per Kg., respectively, for a total of 28 doses. The sections were fixed in formalin and stained. These showed no histological abnormalities. Since intravenous injection provides the most severe test of toxicity, the results indicate that the succinates are not nephrotoxic.

Vomiting and Diarrhea.—The fact that the urine and sections of kidney tissue showed no signs of renal damage left open the question as to the cause of the azotemia resulting from the protracted oral use of the succinates. Hemoconcentration was suggested by the changes shown in Figs. 2 and 3, increased blood count and hemoglobin as well as rise in blood NPN and creatinine. This possibility received support from the observations on vomiting and diarrhea. The incidence of gastrointestinal symptoms are summarized in Table III. It may be noted that vomiting and/or diarrhea occurred with all preparations of succinates after both oral and intravenous administrations. These effects occurred in from thirty minutes to several hours after the doses. The different animals vomited from 3.6% to 33.9% of the doses. Diarrhea was most frequent after the magnesium succinate. The vomiting frequently occurred on the day before the blood samples were taken. It is noteworthy that the greatest elevation of blood NPN (average 43.2 mg.% during control and 72.9 mg.% during treatment) occurred in a cat which received magnesium succinate, developed nearly a daily diarrhea, and showed the highest incidence of vomiting the day before the blood sample was taken (6 of the 11 samples). It seems probable that the increase in blood count and azotemia resulting from the prolonged use of large doses of succinates are due to hemoconcentration.

TABLE III.—INCIDENCE OF VOMITING AND DIARRHEA

Cat No.	Doses, Gm./Kg.	No.	Route	Per Cent of Doses Vomited	Per Cent of Doses Diarrhea
			Sodium Succinate		
1	0.5	75	Oral	29.3	2.3
2	0.5	68	Oral	33.9	1.5
			Magnesium Succinate		
3	0.5	68	Oral	29.3	54.4
4	0.5	68	Oral	14.7	82.3
			Succinic Acid		
5	0.5	68	Oral	13.2	0
6	0.5	68	Oral	11.8	4.4
			Sodium Chloride		
8	0.5	28	Oral	7.2	0
9	0.5	28	Oral	0	0
			Sodium Acetate		
12	0.5	28	Oral	7.2	0
			Sodium Succinate		
10	0.5	28	Intravenous	10.7	0
11	1.0	28	Intravenous	3.6	0

SUMMARY

The present report deals with a study of the effects of succinic acid, sodium succinate, and magnesium succinate in cats. Observations were made after single intravenous and oral doses, and during the use of daily intravenous and oral doses for periods of from one to nearly three months. The study included the effects on the general behavior of the animals, the nutritional state, blood pressure, gastrointestinal tract, liver function, blood, and kidney function.

The results show that large doses of these compounds produce numerous acute disturbances, vomiting and diarrhea, fall of the blood pressure, convulsions, and death. Vomiting results after intravenous doses as small as 0.25 Gm. per Kg., and vomiting and/or diarrhea is frequent after oral doses of 0.5 Gm. per Kg. An intravenous dose of about 2 Gm. of sodium succinate per Kg. may produce abrupt fall of arterial pressure and death, and an oral dose of 5 Gm. per Kg. may cause death in convulsions with primary cessation of respiration especially if vomiting is impaired by a small dose of morphine. It is noteworthy that morphine does not completely suppress the vomiting or diarrhea induced by these salts. The diarrhea seems to be more conspicuous in the case of the magnesium salt, probably because of the poorer absorption.

The protracted administration of better-tolerated oral doses, 0.5 Gm. per Kg. daily, for several weeks appears to produce no general signs of poisoning or impairment of nutrition, and no toxic effects on the blood, the liver, or the kidney. A rise of the blood count and azotemia occur, apparently as the result of hemoconcentration, the consequence of repeated vomiting and diarrhea.

The causes of the marked disturbances produced by large doses of the succinates, seem to be nonspecific. Isotonic solutions produce no effects until the water content of the dose amounts to several times the blood volume of the animal. Hypertonic solutions produce effects indistinguishable from equitonic solutions of sodium chloride. Large doses of the more soluble sodium succinate produce effects indistinguishable from similar doses of sodium bicarbonate. Any specific toxicity which the succinate ion

may possess is therefore masked by the effects of excessive fluid, hypertonicity, acid, or alkali. The indications are that the succinate ion is substantially inert, and doubt remains as to whether it exerts any specific toxic effects.

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Laxative Action of Succinates in Man*,†

By LEON J. WARSHAW and HARRY GOLD

The laxative effect of 260 10-Gm. doses of succinic acid, sodium succinate, and magnesium succinate administered to 30 patients suffering with chronic constipation is reported. No nephrotoxic action was noted upon prolonged administration. A dosage form is suggested to overcome the unpleasant effects sometimes experienced by the ingestion of simple solutions of succinates in water.

IN A PREVIOUS study (1), a method was described for the evaluation of laxative agents in constipated human subjects. This method and modifications of it were used in observations on the laxative properties of gluconates (2), fumarates, tartrate, and citrate (1, 3). Several carboxylic acids and their salts are known to possess laxative properties, and some of them, notably tartrates, are nephrotoxic. There are several isolated observations indicating that the succinates possess little, if any, toxicity in man, and a recent study in cats (4) shows that succinic acid, sodium succinate, and magnesium succinate are substantially free of toxic actions which cannot be ascribed to excessive fluid, acidity, or alkalinity. This study shows that large doses of these compounds, the equivalent of about 30 Gm. for a man, frequently produce vomiting and/or diarrhea in cats.

The present report deals with the results of a study designed to test the laxative properties of the succinates in constipated ambulant human subjects. There are also observations relating to the toxicity of the succinates in man.

EXPERIMENTAL

The subjects used were ambulant adult patients who had been in regular attendance at a cardiac clinic for periods varying from several months to several years. Many of these patients required maintenance doses of drugs for their cardiac condition but only those whose heart disease and state of compensation were relatively stable were selected. The basis for their assignment to this study was functional constipation with a long history of dependence upon laxatives. The agents that had

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† This study was aided in part by a grant from the Monsanto Chemical Company.

been used were Epsom salts, Seidlitz powders, cascara, milk of magnesia, or phenolphthalein preparations. The character of the constipation was such that a laxative was found necessary at least twice weekly, some patients having become accustomed to a daily dose of a laxative. When they failed to take it, several days would elapse without a bowel movement.

water followed by one-half glassful of water. The plan was to have the drug approximately isotonic in the gastrointestinal tract.

A simple record form was issued at every clinic visit made at intervals of one or two weeks. It contained the following rubrics: Day of the week, time medicine was taken, time of bowel movement, kind of bowel movement, and any other symptoms.

TABLE I.—LAXATIVE EFFECTS OF SUCCINATES

	Succinic Acid	Sodium Succinate	Magnesium Succinate	Total
No. patients	24	10	8	30[a]
No. doses	128	100	32	260
No. "positive results"	70 (54%)	84 (84%)	26 (81%)	180 (69%)
Time to effect				
Average	9.8 hr.	6.0 hr.	5.4 hr.	....
Range	1–24 hr.	1–24 hr.	1–11 hr.	....
Vomiting				
No. patients	8 (33%)	4 (40%)	4 (50%)	14[a] (47%)
No. doses	15 (12%)	14 (14%)	6 (19%)	35 (13%)

[a] These numbers are smaller than the sum of the columns because some patients were used for more than one drug.

TABLE II.—COMPARISON OF SUCCINATES IN THE SAME PATIENT

Patient	J. T.	M. S.	S. Y.	F. S.	M. G.	M. B.	F. L.	B. K.
Succinic Acid								
Dose, Gm.	10	10	5, 10	10	10	10	10	..
No. doses	14	2	5 each	3	9	2	6	..
No. "positive responses"	9	1	10	1	9	1	5	..
Time to response, hr.	17 (4–23)	5	5 (2–12)	3	5 (1–12)	..	8 (5–12)	..
Vomiting, No. doses	0	1	0	0	1	0	0	..
Sodium Succinate								
Dose, Gm.	10	10	10	10	10	10	10	10
No. doses	5	1	10	4	22	5	17	29
No. "positive responses"	4	1	9	0	22	5	17	26
Time to response, hr.	23 (22–24)	9	6 (1–18)	0	3 (2–9)	3 (2–4)	2 (1–8)	8 (2–14)
Vomiting, No. doses	0	0	0	4	0	0	0	3
Magnesium Succinate								
Dose, Gm.	..	10	10	10	10	..	..	10
No. doses	..	3	2	2	3	..	..	8
No. "positive responses"	..	3	2	0	3	..	..	8
Time to response, hr.	..	8 (7–8)	2 (1–2)	0	4 (3–4)	..	..	10 (7–11)
Vomiting, No. doses	..	0	0	2	1	..	..	0

The patients were instructed to take the agent under investigation, following the lapse of at least twenty-four hours after the expected daily bowel movement. In the majority of cases, it was taken in the morning. If twenty-four hours then elapsed without a bowel movement, the dose was presumed to have failed and the patient was instructed to take a soap-suds enema. The dose was not repeated for at least forty-eight hours following the enema, since an enema itself often interferes with the subsequent action of a laxative.

The compounds—succinic acid, sodium succinate, and magnesium succinate—were dispensed in powders containing 10 Gm. each, in a few instances 5 Gm. The dose was taken dissolved in one glass of

The patient filled out this form for each dose, and returned it during the subsequent clinic visit. A large proportion of the patients were familiar with this technic of observation and recording, having served as subjects in previous studies of this type.

RESULTS

Table I summarizes the results with the entire group of 260 doses in 30 patients. The number of doses taken by the individual patient varied from 1 to 37, average 6 doses. All the patients developed a laxative effect after one dose or another, and such a result occurred after nearly three-fourths of the doses in an average period of seven hours with wide variations of from one to twenty-four hours.

Minor unpleasant symptoms were very frequent, nausea, epigastric discomfort, diarrhea, and abdominal cramps. Vomiting was produced in about one-half of the patients and after about one-sixth of the doses. No conspicuous difference was in evidence among the 3 preparations with respect to laxative activity or disagreeable symptoms, the differences seen in the table being probably due to the relatively small numbers and wide variations in individual responses.

In Table II are summarized the results with 157 doses in 8 patients who received either 2 or all 3 preparations. It may be noted that in these the incidence of laxative responses and of vomiting are of the same order for each of the 3 preparations.

While there are some suggestive observations in the literature indicating a mild nephrotoxic action of succinates in rabbits, the study in cats (4) failed to disclose any such action after very large doses. In 9 subjects who received from 4 to 37 oral doses of 10 Gm. each of the succinates, the urine was examined for specific gravity, albumin, casts, and blood cells, and the blood for nonprotein nitrogen. All 3 preparations were represented. A specimen was taken before the study was started and again after the last dose. There were no significant changes in the urine. The results of the blood NPN values are summarized in Table III. While the values are sometimes higher after the drug than before, the reverse is often the case, and there is no significant trend.

TABLE III.—EFFECT OF SUCCINATES ON BLOOD NPN

Patient	No. Doses	NPN, Mg.% Before	NPN, Mg.% After
B. K.	9	39	41
J. B.	10	39	40
J. T.	19	32	34
B. M.	13	31	35
S. Y.	22	37	34
M. G.	34	42	29
B. R.	13	31	46
L. G.	4	29	36
B. K.	37	43	30

The unpleasant symptoms appear to be due to local action involving taste and irritation. Succinic acid is quite sour; sodium succinate produces an immediate sense of coolness and is faintly bitter; magnesium succinate produces an immediate sense of heat with a stronger bitter taste and appears to be the most unpleasant of the 3. The dose of 10 Gm. used in most cases of this study appears to be larger than the average required dose since all patients responded at one time or another, and the laxative response for the total number of doses was 69%. In the practical application of the succinates as laxative agents, smaller doses to test the patient's sensitivity, larger volume of water with each dose, or a preparation in an effervescent form, may help to solve the problem of unpleasant reactions.

SUMMARY

Succinic acid, sodium succinate, and magnesium succinate, administered in doses of 10 Gm. to 30 patients suffering with chronic constipation, produced a laxative effect after an average period of about 7 hours in approximately three-fourths of 260 doses tested. The prolonged use of such doses of succinates appeared to be without nephrotoxic action in man as judged by the urine and blood NPN. Such doses adminsistered in one and one-half glassfuls of water caused frequent unpleasant effects including vomiting. No significant difference with respect to laxative activity or disagreeable side effects among the 3 preparations was in evidence. A suggestion is made concerning a possible means for controlling the unpleasant effects in the practical application of the succinates as laxative agents.

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Notes on the Pharmacology of Sodium Fluoroacetate —Compound 1080*,†

By JUSTUS C. WARD and D. A. SPENCER‡

Results of extensive lethal dose studies on rats, mice, ground squirrels, prairie dogs, cats, bobcats, dogs, coyotes, badgers, guinea pigs, sheep, cattle, horses, chickens, hawks, eagles, and other forms of life are presented. Descriptions of physiological reactions shown by the various species to the poison are given. Comparative toxicities of baits made with sodium fluoroacetate, thallium sulfate, white arsenic, zinc phosphide, and barium carbonate, as well as strychnine alkaloid, are shown. Secondary poisoning hazards to the predatory animals most susceptible to this poison are described. The utility of sodium fluoroacetate as an economic poison is discussed.

FOLLOWING the discovery in 1944 that sodium fluoroacetate was an effective poison for use in the control of certain animal pests, several popular articles appeared describing its general characteristics and outlining methods of use (1–6, 8–10).

All of the early releases emphasized the fact that this new poison was highly toxic, not only to the undesirable species, but also to pets, livestock, beneficial wildlife, and human beings. The articles summarized the hazards of use, evaluated them in relation to the improved results which employment of 1080 produced, and then concluded that this new poison should be entrusted only to persons experienced in handling highly toxic substances in animal control.

Since the utility of the poison was discovered during the war, and inasmuch as it was found to be an effective substitute for certain of the critically short materials needed in rodent control, it was essential to permit limited distribution of 1080 before a great deal of its basic pharmacology could either be worked out or published. That deficiency is being corrected (7, 11–13); and this paper is a contribution from this laboratory on the subject of the acute toxicity of this new poison. Future releases will deal with some of the more technical phases of its action.

In determining the value of 1080 in the control of all noxious rodents, it was important to run several experiments on related species of animals. The data presented in the experimental section of the paper, therefore, show the acute toxicity for rodents, predatory animals, and certain birds. Records taken from information released by the Chemical Warfare Service (7) are properly credited to that agency.

DISCUSSION

Early studies on the compounds containing fluoroacetic acid indicated that the route of administration had slight effect on the LD_{50} dose. This was notably true of the methyl ester (7). It is noteworthy to point out that this observation is not entirely true with regard to the sodium salt. Chenoweth and Gilman (7) emphasize the extreme variation in susceptibility to fluoroacetate poisoning shown by different animals. They indicate, also, that death may result from cardiac difficulty, central nervous system depression, or a combination of the two. Gross symptoms observed in the animals used in these experiments confirm the variable cause of death. That fluoroacetates are extremely dangerous from the secondary, as well as from the primary poisoning angle, has been emphasized by several authors (2, 3, 5, 6, 9–11).

Since high toxicity is one essential characteristic in the most useful economic poisons, it is apparent from the data presented that sodium fluoroacetate should prove to be a very effective one. That reasonable safety in use is a prerequisite for any toxic agent which is to be released for sale to the general public is self-evident. The most casual review of the tables in the experimental section indicates that 1080 is dangerous to beneficial animals and to man. It is another poison for which there is no effective antidote. Its distribution must be restricted to experts who will use it sanely.

Further examination of the results obtained in the acute toxicity experiments reported in this paper indicates that sodium fluoroacetate is a unique substance—from the pharmacological view-

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† A portion of this work was carried out with funds provided by the Office of Scientific Research and Development.
‡ We are pleased to acknowledge the cooperation of Mr. Howard A. Merrill and Mr. H. J. Spencer in gathering some of the data presented.

TABLE I.—ACUTE LETHAL DOSES OF SODIUM FLUOROACETATE

Animal Name	No. Used	Route of Administration	LD_{100}%, Mg./Kg.	*LD* Figures Below 100%, Mg./Kg.	
Field Rodents					
Ground Squirrels:					
Apache spotted (*Citellus spilosoma canescens*)	9	IP[a]	0.5	LD_{50}	0.4
Columbian (*Citellus columbianus columbianus*)	52	IP	0.3	LD_{50}	0.1
				LD_0	0.05
Fisher (*Citellus b.* Fisheri)	180	Fed	0.35	...	...
Pocket Gophers:					
Breviceps—Texas (*Geomys breviceps* sp.)	9	IP	0.05	....	....
Tuza—Florida (*Geomys floridanus*)	26	ST[b]	0.5	LD_{50}	0.25
Kangaroo Rats:					
Bannertail (*Dipodomys s. spectabilis*)	63	IP	0.2	LD_{67}	0.1
Merriam (*Dipodomys m. merriami*)	27	IP	0.3	LD_{85}	0.2
				LD_0	0.1
Fresno (*Dipodomys nitratoides*)	25	Fed	1.0[c]	...	....
Rats:					
Cotton—Florida (*Sigmodon hispidus littoralis*)	35	ST	5.0[d]	LD_{50}	0.1
Wood rat—California (*Neotoma intermedia*)	17	Fed	2.0	LD_{50}	1.0
Norway—Florida (*Rattus r. norvegicus*)	31	ST	8.0	LD_{75}	4.0
	17	Fed	...	LD_{71}	4.0
Alexandrine (*Rattus r. alexandrinus*)	60	ST	2.0[c]	...	....
	33	Fed	2.0	LD_{92}	1.0
Wood rat—Arizona (*Neotoma a. albigula*)	44	IP	0.8[c]	...	....
Wild mice:					
Pocket (*Perognathus i. inornatus*)	7	Fed	1.0[c]	...	....
Meadow (*Microtus haydeni*)	35	Fed	0.5	LD_{33}	0.3
Deer (*Peromyscus* sp.)	54	Fed	5.0	LD_{50}	4.0
				LD_{29}	2.0
House (*Mus musculus*)	61	ST	...	LD_{64}	10.0
Prairie dogs:					
Plains (*Cynomys ludovicianus*)	15	ST	0.3[c]	...	...
	9	Fed	0.9[c]	...	...
Porcupine:					
Western (*Erethizon epixanthum*)	3	IP	1.0	...	....
Laboratory animals					
Rats:					
White	24	Fed	...	LD_{50-3}	2.5
	45	IP	7.5	LD_{50}	3.0–5.0
Mice:					
White	190	IP	40.0	LD_{95}	20.0
				LD_{16-6}	10.0
Guinea pigs	24	Fed	0.5	LD_{44}	0.4
Wild carnivores					
Coyotes (*Canis latrans nebracensis*)	22	IP	0.2	LD_{50}	0.1
Bobcats (*Lynx rufus baileyi*)	5	IP	0.66[c]	...	...
Badgers (*Taxidea taxus berlandieri*)	6	IP	1.5	LD_0	1.0
Grey fox (*Urocyon cinereoargenteus scotti*)	4	IP	0.3[c]	...	....
Carrion-feeding birds					
Golden eagle (*Aquila chrysaetos*)	8	Fed	...	LD_{50}	5.0[e]
American rough-legged hawk (*Buteo lagopus sancti-johannis*)	6	Fed	...	LD_{50}	10.0[e]
Ferruginous rough-legged hawk (*Buteo regalis*)	2	Fed	...	LD_{50}	10.00[e]
Marsh hawk (*Circus hudsonius*)	4	Fed	...	LD_{50}	10.0[e]
Great Horned Owl (*Bubo virginianus pallescens*)	5	Fed	...	LD_{50}	10.0[e]
Turkey Vulture (*Cathartes aura*)	7	ST	...	LD_{71}	20.0
Black Vulture (*Cartharista urubu*)	10	ST	...	LD_{50}	15.0
	333	Fed	...	LD_{11}	50.0
Magpie (*Pica p. hudsonica*)	12	Fed	1.3	LD_0	0.67

(*Continued—next page*)

TABLE I.—ACUTE LETHAL DOSES OF SODIUM FLUOROACETATE (*Continued*)

Animal Name	No. Used	Route of Administration	LD_{100}%, Mg./Kg.	*LD* Figures Below 100%, Mg./Kg.	
Game birds					
Gambel's quail (*Lophortyx gambelii*)	44	Fed	...	LD_{57}	20.0
Mourning dove (*Zenaidura macroura*)	30	Fed	...	LD_{25}	10.0[e]
Passerine birds					
Brewer's Blackbird (*Scolecophagus cyanocephalus*)	12	Fed	...	LD_{33}	2.0–3.0
English sparrow (*Passer domesticus*)	47	Fed	...	LD_{43}	2.0–3.0
Wild ducks					
Mallards (Males)	17	ST	7.5	LD_{75}	7.0
Mallards (Females) (*Anas boschas*)	18	ST	16.0	LD_{75}	15.0
Pintails (Males)	12	ST	...	LD_{50}	10.0
Pintails (Females) (*Dafila acuta*)	8	ST	10.0	LD_{50}	8.0
Widgeon (Males)	8	ST	4.0	LD_{50}	3.0
Widgeon (Females) (*Mareca americana*)	8	ST	11.0	...	....
Domestic poultry					
Rhode Island Red hens	12	Fed	...	$LD_{83.3}$	6.5
Plymouth Rock hens	12	Fed	...	LD_{58}	5.9
White Leghorn chickens	24	Fed	...	$LD_{83.3}$	17.7
				$LD_{8.3}$	8.85
Pigeons—Florida	29	ST	...	$LD_{67.5}$	10.0
Pigeons—Colorado	18	Fed	5.0	LD_{50}	2.0–3.0

[a] Intraperitoneal injection.
[b] Stomach tube administration.
[c] All animals died at dose indicated (smaller doses not given).
[d] Erratic response.
[e] Emesis a characteristic and early sympton.

point. The authors trust that this fact will appeal to workers in other laboratories. To stimulate fundamental research with this particular chemical, the Wildlife Research Laboratory will be in position to furnish small lots of sodium fluoroacetate to pharmacologists who might be interested in studying specific phases of its action.

EXPERIMENTAL

The lethal doses listed in the preceding table were determined by staff members of this Laboratory working in Denver, Colo., Gainesville, Fla., and on field assignments in many sections of the United States.

Edgewood Arsenal data (LD_{50} of methyl fluoroacetate by any route) (7) is as follows:

Animal	Dose in Mg./Kg
Dog	0.06
Rabbit	0.22
Guinea pig	0.35
Pig	0.40
Cat	0.50
Goat	0.60
Horse	0.50–1.75
Hamster	2.50–5.00
Rhesus monkey	4.00
White rat	5.00
Spider monkey	15.00

It is apparent that there are many gaps in the table, which may be explained by pointing out that it was found to be unnecessary to obtain complete data on all of the species of animals and birds which were studied. The symptoms of poisoning shown by the animals used in our experiments varied from a progressive depression, illustrated by bobcats and Columbian ground squirrels, to most violent epileptiform convulsions, which were characteristic of badgers, coyotes, and fisheri ground squirrels. The reasons for this peculiar difference between two species of ground squirrels should be sought.

CONCLUSIONS

1. The acute toxicity of sodium fluoroacetate for 25 different forms of mammals and 19 kinds of birds are presented. For mammals the LD_{100} figures vary from 0.05 mg./Kg., determined for the pocket gopher in Texas, to 40.0 mg./Kg. as found for the laboratory white mice, with all doses on the IP basis. With birds, the LD_{100} figures are not available, but the approximate LD_{50} doses vary from 2.0 mg./Kg. on pigeons to above 50.0 mg./Kg. on black vultures on the "Fed" basis.

2. Distinct differences in symptoms of poisoning are shown by various species.

3. There is a variation in susceptibility of ducks to sodium fluoroacetate due to sex, with male mallards and widgeon being more sensitive than females.

4. Edgewood Arsenal data indicate that

primates are relatively resistant to the action of the fluoroacetates, but that the actual toxicity is great enough to justify the warning that these compounds must be handled with extraordinary caution.

5. A great deal more study on sodium fluoroacetate is needed. The Wildlife Research Laboratory offers cooperation to other laboratories wishing to undertake experiments with this compound.

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The Colorimetric Determination Of Bismuth in Pharmaceutical Preparations and in Biological Material*

By GEORG CRONHEIM† and PAUL H. WRIGHT

A colorimetric method of general applicability for the determination of bismuth is described. The method makes use of the relatively stable yellow color of the complex formed when thiourea reacts with bismuth.

THE ASSAY of bismuth in pharmaceutical preparations presents usually no particular difficulties. However, in many cases the necessary separation from other ingredients is rather time consuming and cumbersome. Therefore, it was desirable to develop a quick and at the same time reliable procedure for the determination of bismuth, and it was felt that a colorimetric method would best answer this purpose.

A survey of the literature indicated that the reaction of bismuth with thiourea first discussed by Hoffman and Gonder (1) was the most promising. It results in a strongly colored yellow solution which follows Beer's law within fairly wide limits (Mahr, 2). According to West and Tokos (3) the only positive interferences are produced by antimony (III), palladium (II) and vanadic and tellurous acid, none of which is commonly combined with bismuth in pharmaceutical preparations. Mercury and copper, if present in large amounts, form a precipitate with thiourea and thus can be filtered off. It is also recommended by Mahr (2) to reduce trivalent iron with hydrazine sulfate to the divalent form. In our own experiments we could not find any indication that this step is necessary.

The yellow color of the bismuth-thiourea complex is formed immediately, and it is stable for several hours. In order to obtain reproducible results it is necessary that the final solution contains at least 5 per cent and preferably from 6 per cent to 7 per cent (*w/v*) of thiourea. The maximum absorption lies at about 460 to 465 mμ. It is reported by Mahr (2) that in the presence of larger amounts of chloride ions the maximum absorption is shifted toward shorter wave lengths.

* Received July 31, 1946, from the Laboratories of The G. F. Harvey Company, Saratoga Springs, N. Y.
† Present address: The S. E. Massengill Company, Bristol, Tenn.

The sensitivity of the thiourea reaction for bismuth is far greater than is required for pharmaceutical assays. Using a photoelectric colorimeter with a 30 mm. absorption cell the limit of the method is about 8 micrograms of bismuth in 25 ml. For general assay purposes it has been found that it is most practical to use solutions containing 0.5 to 1.0 mg. of bismuth in 25 ml. This calibration is well within the range of Beer's law. Figure 1 shows a calibration curve using a Lumetron Photoelectric Colorimeter (Model 402-EF) with a 10-mm. cell and a light filter with maximum transmission at 465 mμ.

EXPERIMENTAL

Reagents.—Thiourea Solution: Dissolve 10 Gm. of thiourea in 100 ml. of approximately normal nitric acid. This solution is stable for one to two weeks if kept in the dark. If a sediment of precipitated sulfur is present, it should be filtered before use.

Normal Nitric Acid (approximately): Dilute 70 ml. of nitric acid C. P. (sp. gr., 1.41) to 1000 ml.

Nitric Acid, C. P. (sp. gr., 1.41).

Nitric Acid, 10%.

Hydrochloric Acid, C. P.

Hydrogen Peroxide, 30% solution. (Superoxol)

Ten milliliters of the filtrate are transferred to another 100-ml. volumetric flask and diluted to volume with normal nitric acid.

In the case of an oil solution or a suspension in oil, the sample is dissolved or mixed with chloroform or ether, and the bismuth is shaken out two or three times with 10 ml. of 10% nitric acid. The acid bismuth solution is heated to remove all traces of the organic solvent and the dilution is made as described above.

Colorimetric Determination.—Exactly 5 ml. of the bismuth solution in normal nitric acid are transferred to a 25-ml. volumetric flask and enough of the thiourea solution is added to bring to volume.

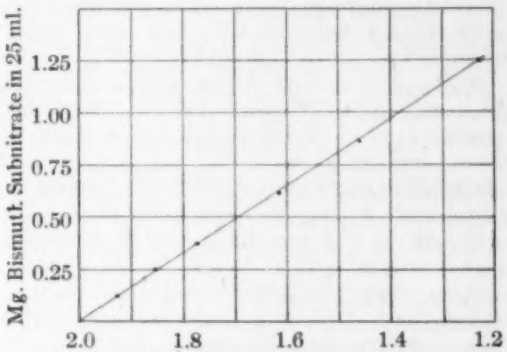

Fig. 1

TABLE I.—AMOUNTS OF BISMUTH IN PERCENT OF LABELED STRENGTH IN VARIOUS PHARMACEUTICAL PREPARATIONS

Preparation	Active Ingredients	Chemical Assay	Colorimetric Assay
Tablet	Bi-subnitrate	100.3	100.1
Tablet	Bi-subnitrate, Ce-oxalate	98.6	99.0
Tablet	Bi-subnitrate, Ca-carbonate Mg-carbonate	100.5	99.8
Tablet	Bi-subgallate, Zn-phenolsulfonate β-Naphthol, Cu-arsenite	99.1	99.8
Suspension	Bi-subgallate, Zn-phenolsulfonate Salol, Lactated Pepsin, Malted Milk	99.2	99.7
Suspension	Bi-subsalicylate, Zn-phenolsulfonate Salol, Kaolin, Lactated Pepsin	101.3	101.6
Ampuls	Bi-subsalicylate in Olive Oil	100.9	100.2
Ampuls	Bismuth and Sodium Tartrate	101.1	101.0

Procedure for Pharmaceutical Preparations

Preparation of Sample.—If the bismuth salt is in a tablet or liquid (suspension) a sample containing 100 to 200 mg. bismuth is transferred to a 100-ml volumetric flask and 15 ml. of nitric acid and 15 ml. of distilled water are added. In most cases the mixture will heat up sufficiently to bring all the bismuth into solution. In the presence of substances which are insoluble in nitric acid, as for instance kaolin, it is advisable to heat the mixture for fifteen to twenty-five minutes on a water bath to assure complete solution of the bismuth. The mixture is cooled, brought to volume, and filtered if necessary. After thorough mixing the solution is ready for the colorimetric determination.

If the final solution of the bismuth salt before the addition of thiourea is colorless no blank determination is required. However, if the original sample contains organic compounds, particularly proteins or protein derivatives, yellow decomposition products will be formed by the action of the nitric acid and therefore a blank determination has to be made. For this purpose 5 ml. of the final dilution are mixed with exactly 20 ml. of normal nitric acid and measured in the colorimeter. The amount of bismuth corresponding to this blank reading is then subtracted

from the amount found in the complete bismuth determination.

In Table I are summarized the results of bismuth determinations in various pharmaceutical preparations. It can be seen that the colorimetric method gives practically the same values as the usual chemical method.

Procedure for Biological Material

The colorimetric determination of bismuth with thiourea lends itself very well to the estimation of bismuth in biological material. Although this method is not quite as sensitive as that with brucine or potassium iodide, it has the great advantage of high selectivity. By a slight modification of the above-described technique it is thus possible to estimate bismuth directly in the ash of biological material.

The minced tissue (5 to 20 Gm.) is dried in a Vitreosil crucible at 100°. The crucible is then transferred to an electric furnace, slowly heated to about 800° and kept at this temperature until all organic material is oxidized. To the residue are added 3 ml. of nitric acid and 2 ml. of hydrochloric acid, and the acid mixture evaporated on a water bath. If there is any question as to complete oxidation the procedure may be repeated with 1 ml. of concentrated nitric acid and a few drops of Superoxol. The ash is then dissolved by boiling for a few minutes with 10 ml. of normal nitric acid. After cooling, the solution is filtered into a 25-ml. volumetric flask. To guarantee complete transfer of the ash from the crucible the procedure is repeated twice with 5 ml. of normal nitric acid each. The solution is then brought to volume with normal nitric acid and well mixed. It is usually slightly colored from the presence of ferric salts and it is therefore necessary to obtain a blank reading in the colorimeter. The solution is then returned to the volumetric flask and 2 Gm. of thiourea crystals are added. After the thiourea is completely dissolved the solution is filtered and measured again in the colorimeter.

The addition of 2 Gm. of thiourea to 25 ml. of the solution increases the volume to about 26 ml. Therefore, it is essential that the standard is treated in exactly the same manner as the unknown or that a volume correction is calculated into the final result,

TABLE II.—RECOVERY OF BISMUTH FROM TISSUE

Amount of Tissue, Gm.	Bismuth Added, μg	Bismuth Recovered	
		μg	%
8	23	18.8	82
12	23	19.5	85
16	23	18.4	80
20	23	21.2	92
24	23	20.0	87

In Table II are summarized the results of a few tests in which 30 micrograms of bismuth subnitrate (23-micrograms of metallic bismuth) were added to various amounts of glandular tissue. It can be seen that the recovery is from 80% to 90%. Apparently, a small part of the bismuth is volatilized during the oxidation of the material. Possibly this might be avoided if a wet ashing process with fuming nitric acid and Superoxol is used (Cronheim, 4).

SUMMARY

A colorimetric method for the determination of bismuth with thiourea in pharmaceutical preparations is described. The procedure is very simple and fast and has the advantage of being practically specific for bismuth.

The method is also applicable to the estimation of bismuth in biological material.

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